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DISTRIBUTION OF RUBBER AND RESINS IN GUAYULE

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(WITH ONE FIGURE)

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Introduction

Rubber contents are so frequently the focus of attention in investigations of guayule (*Parthenium argentatum* Gray) that an account of rubber distribution in the plant may contribute to experimental studies as well as to purely descriptive literature concerning the plant. In the choice of plant material for study or for sampling, or in the interpretation of results, the relative amounts and concentrations of rubber in the various parts may be a pertinent factor. Even commercial scale harvesting and milling operations are aided to some degree by knowledge of which plant parts contribute significantly to rubber yields and which are insignificant or mere diluents.

The pattern of rubber distribution as revealed qualitatively under the microscope has been thoroughly studied and described by Ross (**8**), Lloyd (**6**) and Artschwager (**1**). In plants older than one year, the vascular rays of phloem and xylem carry the major portion of the rubber. Other parenchymatous tissues, the pith, primary cortex, epithelial cells of the resin canals, and xylem parenchyma contain rubber; but, partly because they comprise a smaller fraction of the plant, the amount of rubber they contribute is less important. In younger plants, some of these latter tissues contribute a relatively larger part of the total rubber. Rubber is evident in the leaf parenchyma, but only in very small amounts.

Only incomplete information is to be found concerning the macro-, but quantitative distribution of rubber in guayule. Whittlesey (**13**) separated several portions from native plants of uncertain but evidently advanced age. As determined by carbon tetrachloride extraction, following extraction with alcohol and water, the bark of the root and that of the trunk yielded approximately 20% rubber. Practically no rubber was obtained from the wood of these parts. This supported an impression, already current at that time, that the rubber in guayule is largely restricted to the bark. Above the trunk, which was apparently the sole remnant of the basal branching system typical

of a vigorous young cultivated plant, the leafy branches were analyzed as a composite sample. Aside from Whittlesey's report the literature offers little but fragmentary information, rarely accompanied by published data. Unpublished data from a preliminary survey of a few plants by H. P. Traub and B. J. Cooil indicated a general distribution between bark and wood and between root and top not unlike that shown in the following pages.

Olson[1] separated plants into top and basal portions. From a series of such separations in which the division was at various levels, he obtained indications that the maximum concentration of rubber was in the lowermost (proximal) parts of the branches. This obtained for recovery by milling procedures as well as by direct chemical analyses, and the quality of rubber yielded also seemed better in the lower parts of the branches than in either the root portions or the youngest upper branches.

Materials and methods

Plant material was obtained from various plantations and experimental areas of the Emergency Rubber Project in the vicinity of Salinas, California. The history of the plants prior to field culture was that of the usual planting procedure. Seedlings produced in thickly planted, irrigated nursery beds were transplanted to the field in the fall or winter, after less than a year of growth from seed. The indicated age of the plants refers to the years of growth in the field following transplanting, although the oldest tissues in the root and crown, which were carried over in the transplant, were of course nearly a full year older. Five plants were combined to provide the material for each separation, and the recorded values are the means of six such groups.

The general pattern of subdivision and designation of parts is illustrated (fig. 1). The root system was detached where constriction begins below the swollen crown portion. The short lengths of secondary and finer roots obtained as the plants were dug were removed as a separate sample of branch roots. This represents only a minute part of an extensive branching root system (7), but perhaps more than is removed in the normal harvesting operation of undercutting. The primary root, devoid of the lateral roots, represents approximately the root of the original transplant. An arbitrary decision placed the upper limit of the crown at the level where the few enlarged branch bases gave rise to the more profuse main branching system. Usually the plane of the soil surface passed through the crown region. Above the crown, the branches were separated into the stem lengths corresponding to the successive annual increments of growth. The designations "first year stem, second year stem," *etc.*, accordingly indicate the year of growth after transplanting in which the primary tissues of the stem segments were formed. Detection of the successive stem segments is made possible by the shortened internodes which appear as cessation of bud growth occurs in

[1] OLSON, D. S. Distribution and quality of rubber milled from plant fractions. Unpublished report in the files of the Emergency Rubber Project, Salinas, California. July 14, 1944.

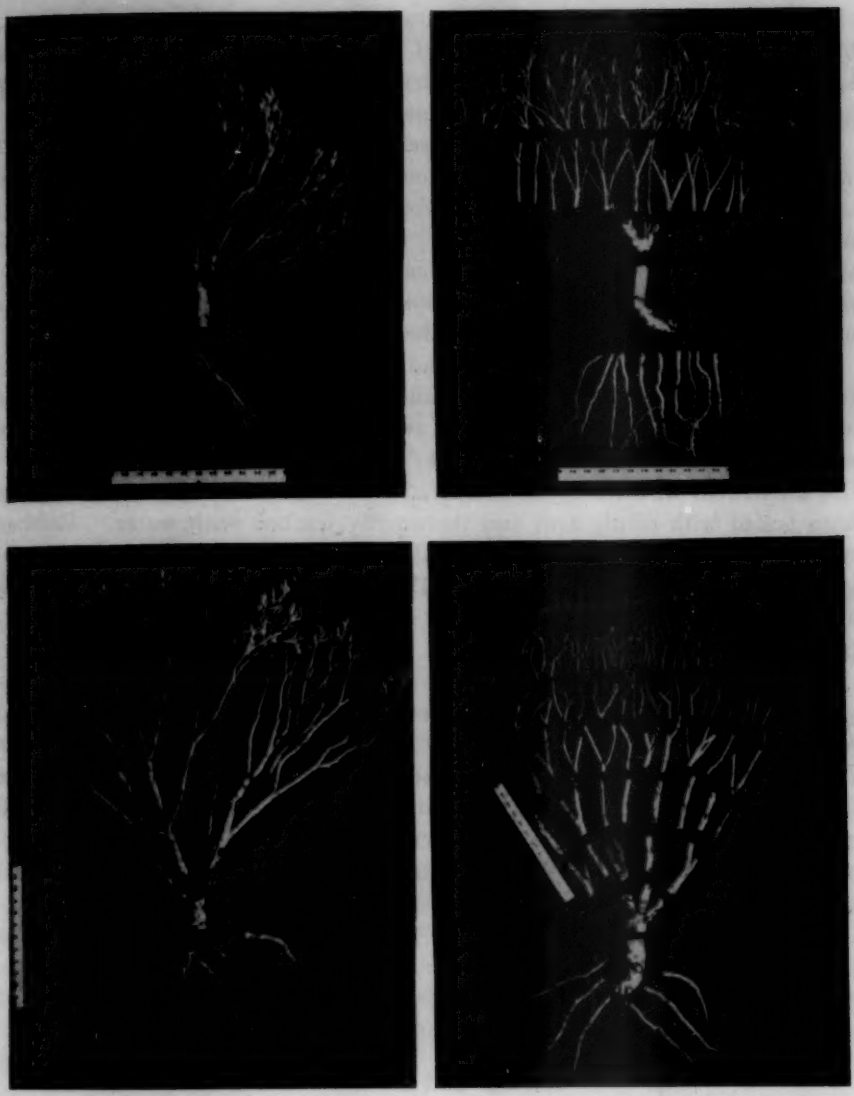

FIG. 1. Appearance of guayule plants as taken from the field (left) and the segments into which defoliated plants were separated (right). Above is a two-year-old irrigated plant showing segments branch roots, root, crown, 1st year stem, and 2nd year stem in upward sequence from the base. Below is a nine-year-old plant and the segments branch roots, root, crown, 1st year stem, 2nd year stem, 3rd and 4th year stem (combined), 5th and 6th year stem (combined), 7th and 8th year stem (combined), and 9th year stem. The rule appearing in each photograph is 12 inches (30.5 cm.) long.

response to exhaustion of available soil moisture or low temperature. Cycles of growth are consequently separated by regions in which the shortened internodes leave a collar of leaf scars similar to the bud scars occurring on deciduous woody plants. The annual increments of growth in length are

clearly distinguishable on young plants growing in the vicinity of Salinas; but on plants approaching ten years of age the markings on the older parts become obscure, and in regions where irregular climatic patterns of moisture and temperature occur, annual increments could not be located accurately.

Dead twigs and branches and the remnants of the wiry flower peduncles were removed as a separate sample from the older parts, and the stem of the most recent growth increment was separated from its attached leaves and flower peduncles. The terms "bark" and "wood" refer to the groups of tissues obtained by separation at the cambium region. By hammering the segments of stem lightly, the tissues can be separated where the fragile cells of the cambium region have been crushed.

Following coarse chopping by hand, the various fractions of plant material were dried at 65° C., then ground successively through a Wiley mill and a hammer mill (**9**). Rubber and resins were usually determined by the method of SPENCE and CALDWELL (**11**). The term "resins" thus refers to the substances which may be extracted with acetone after the sample has been boiled with dilute acid and thoroughly leached with water. Rubber was extracted with benzene following the removal of the acetone-soluble substances. Other methods for rubber (**12, 14**) were employed in special cases as indicated in the text. Percentage contents of rubber and resin are expressed as percentage of dry weight.

The plants of tables I to VI were all sampled in April when growth was just beginning and the rubber concentration was at a maximum which would not be exceeded until the following autumn. In connection with total yields from the plants, it should be mentioned that with plant spacings as wide as those involved in these fields, harvesting is not considered economical until more than three years of growth have elapsed. The plants do not fully occupy the field area until this time, and during the earlier years the annual increments of rubber increase with age.

Statistical analyses was by the method of analyses of variance. From the error variance the standard error of difference between means was ascertained, which by reference to the tables of "t," provided an estimate of the difference required for significance (**5**).

Results and discussion

TWO-YEAR-OLD PLANTS

Table I records the distribution of weight, rubber, and resin among the various segments of root and stem. These plants, taken from the first plantation established in the operations of the Emergency Rubber Project, had grown for two years in the field without any irrigation to supplement the winter rainfall of about 13 inches. Rubber concentration, expressed as percentage of dry weight, is lowest in the root, higher in the branch roots, and much higher in the crown and branches. The oldest segments of the branches, stems formed during the first year, are higher in rubber content than the second year stems; but the difference is surprisingly small in view

TABLE 1

DISTRIBUTION OF RUBBER, RESIN, AND WEIGHT IN TWO-YEAR-OLD GUAYULE
NO IRRIGATION TO SUPPLEMENT WINTER RAINFALL; STRAIN 593; SPACED 36 BY 24 INCHES; SAMPLED APRIL, 1944

PLANT PART	RUBBER IN DRY TISSUE	RESIN IN DRY TISSUE	DRY WEIGHT		WEIGHT OF RUBBER		FRESH WEIGHT PER PLANT
			PER PLANT	FRACTION OF TOTAL†	PER PLANT	FRACTION OF TOTAL†	
	%	%	*gm.*	%	*gm.*	%	*gm.*
Branch roots	8.96	7.66	4.9	3.2	0.44	2.6	12
Root	8.01	6.00	26.3	17.1	2.11	12.6	53
Crown	11.43	6.28	18.3	11.9	2.09	12.5	35
1st year stems*	12.07	6.76	50.3	32.6	6.07	36.3	93
2nd year stems	11.05	8.00	54.3	35.2	6.00	35.9	102
Least sign. 5%	0.71	0.51	.....	1.0	.....	1.4	.....
difference 1%	0.97	0.69	.....	1.3	.....	1.9	.....
Total plant	10.34	7.04	154.1	100	16.71	100	295

* Stem segments are numbered according to sequence of appearance during the plants' growth. Thus the primary tissues of the 1st year stems were formed in the first year of growth.

† Relative quantity in the part is expressed as percentage of total in the plant.

of the fact that the oldest parts of the former are two years old, while those of the latter are only one year old. It is interesting to consider that although the first year stem contained a considerable percentage of rubber (table VII) before the growth of the second year stem even started, the two are now not greatly different in rubber content.

Resin concentration is also at a minimum in the root, but with only an insignificantly higher concentration in the crown. The second year stem, formed during the past year, shows distinctly more resin than the older stem.

The data show that two-thirds of the dry weight of the plant is in the branch system above the crown, about one-third distributed between the root and crown, and only a very minor part in the secondary roots. The composition of the branches will, therefore, be the predominant factor in gross composition of the plant as a whole.

Distribution of the total weight of rubber among the parts is roughly parallel to the distribution of dry weight. The contribution of the root in terms of rubber quantity, however, is less than in terms of dry weight, because of its low rubber concentration; the contribution of the branch system is correspondingly greater.

Plants represented by table II are also two years old, but they had been irrigated in July and September of each year. Detailed comparisons attributed to irrigation treatment, are not to be made between these tables I and II, for the plants are from independent experiments on separate plantations, the spacings in the field are different, and two varieties are involved. The nominally distinct strains "593" and "406," however, are practically indistinguishable in growth and rubber content (**3**), so the sharply contrasted levels of rubber concentration probably reflect principally the contrasting moisture conditions of the plants. It is also probably justifiable to note that although the longer period of summer growth provided by irrigation resulted in a lower rubber concentration throughout the plant, a compensatingly higher dry weight resulted. Consequently, the rubber yield per plant is very similar under the two moisture conditions. Such approximately compensating responses to irrigation have commonly been observed when natural conditions have not been extremely xerophytic (**4**).[2, 3]

Despite the over-all dissimilarity between populations, the relations within the plants of table II are similar to those shown in table I. Rubber concentration is lower in the root than in any other part. Here, however, the branches are significantly higher in rubber concentration than in the crown, and the younger second year stem not only approaches but even equals the first year stem in rubber concentration. Resin concentration is again at a minimum in the root, and the second year stem is richer in resin

[2] HOLMES, RAIFORD L. Analyses of samples from indicator plots taken in February 1944. Unpublished data summarized in the files of the Emergency Rubber Project, Salinas, California. 1944.

[3] TINGEY, D. C. The effect of different frequencies of irrigation on two-year-old guayule. Unpublished report in the files of the Emergency Rubber Project, Salinas, California. March 17, 1945.

TABLE II

DISTRIBUTION OF RUBBER, RESIN, AND WEIGHT IN IRRIGATED TWO-YEAR-OLD GUAYULE
IRRIGATED IN JULY AND SEPTEMBER OF EACH YEAR; STRAIN 406; SPACED 28 BY 24 INCHES IN FIELD; SAMPLED APRIL, 1944

PLANT PART	RUBBER			RESIN			DRY WEIGHT					WEIGHT OF RUBBER				FRESH WEIGHT	
	IN DRY TISSUE			IN DRY TISSUE			PER PLANT			BARK PERCENTAGE	FRACTION OF TOTAL	PER PLANT			FRACTION OF TOTAL	PER PLANT	BARK PERCENTAGE
	BARK	WOOD	WHOLE	BARK	WOOD	WHOLE	BARK	WOOD	WHOLE			BARK	WOOD	WHOLE			
	%	%	%	%	%	%	gm.	gm.	gm.	%	%	gm.	gm.	gm.	%	gm.	%
Branch roots	.....	.....	4.91	.....	.....	6.40	.....	.....	22.7	.....	8.6	.....	.....	1.12	6.7	48	.....
Root	10.01	0.85	3.90	8.40	2.34	4.33	12.8	25.8	38.6	33.2	14.6	1.28	0.22	1.51	9.0	76	43.4
Crown	10.19	2.93	6.12	8.12	2.91	5.22	13.0	16.6	29.6	44.0	11.2	1.32	0.49	1.81	10.8	56	50.2
1st yr. stems	9.72	3.59	7.13	7.90	3.10	5.88	45.2	32.9	78.1	57.9	29.6	4.39	1.18	5.57	33.1	142	62.5
2nd yr. stems	8.95	4.12	7.22	7.91	5.92	7.20	60.5	34.0	94.5	64.0	35.9	5.42	1.40	6.82	40.5	177	68.0
Least sign. 5%	0.43	0.24	0.35	0.64	0.55	0.28	.....	.....	.....	1.3	2.1	.....	.....	.....	2.0	.....	1.4
difference 1%	0.58	0.33	0.48	0.89	0.76	0.37	.....	.....	.....	1.9	2.9	.....	.....	.....	2.7	.....	1.9
Total plant	.....	.....	6.39	.....	.....	6.09	.....	.....	263.5	.....	100	.....	.....	16.83	100	499	.....

than is any other part. The two stem increments together contain about two-thirds of the total dry weight of the plant, and because of their relatively high rubber concentration, nearly three-fourths of the total rubber.

Separation of the various segments into bark and wood portions reveals partial explanations for the pattern of rubber concentration among the whole segments. Of particular significance in this connection is the fact that rubber is concentrated primarily in the bark; also of importance is the fact that the proportion of bark increases progressively from the root upward to the most recent stem segment. Thus, although the root bark is as high in rubber as the bark of any other part, there is proportionally much less of the rubber-rich bark in the root sample. This, coupled with the very low rubber content of the root wood, produces a relatively low rubber concentration for the root as a whole. In the matter of the second year stem's attaining a rubber concentration approaching that of the older first year stem, the bark of the younger stem yields a rubber concentration quite close to that of the older stem. Even this difference is counteracted by the greater proportion of bark in the young stem and to a slight extent by the higher concentration in the wood, so that the resultant concentration of the two stem segments is nearly identical. Rubber concentration of the bark fraction is at a maximum in the root and the crown, somewhat lower in the first year stem, and still lower in the young second year stem. The wood on the other hand exhibits an extremely low rubber content in the root, and the concentration rises progressively through the upper parts of the plant.

Resin, as rubber, finds its site of principal concentration in the bark. It is distributed rather uniformly through the bark of the various segments. A slight gradient upward is suggested in the wood, but the only impressive distinction is in the high resin concentration of the wood in the second year stem. This contributes to the markedly high resin content of the second year stem as a whole; differences in resin concentration among the other whole segments are largely reflections of varying proportions of bark and wood.

When rubber content is considered in terms of the actual amount in the parts, it is seen that the major part is carried in the bark, as would be indicated from the distribution of weight and rubber concentration. Depending on the segment, the amount in the bark comprises from 72% to 85% of the total. Of the total rubber in the plant 79% is carried in the bark tissues. This figure ignores the branch roots, but the undetermined distribution there would not alter the figure greatly, because when included they contain less than 7% of the total rubber observed.

THREE-YEAR-OLD PLANTS

Tables III and IV present data for plants which have grown for three years in the field. All were irrigated once during the first year but not during the second year. In the third year the plants of table III continued without irrigation, while those of table IV received a single irrigation in

TABLE III

DISTRIBUTION OF RUBBER, RESIN, AND WEIGHT IN THREE-YEAR-OLD GUAYULE
IRRIGATED IN FIRST YEAR ONLY; STRAIN 130, SPACED 36 BY 24 INCHES; SAMPLED APRIL, 1944

PLANT PART	RUBBER			RESIN			DRY WEIGHT					WEIGHT OF RUBBER				FRESH WEIGHT	
	IN DRY TISSUE			IN DRY TISSUE			PER PLANT			BARK PERCENTAGE	FRACTION OF TOTAL	PER PLANT			FRACTION OF TOTAL	PER PLANT	BARK PERCENTAGE
	BARK	WOOD	WHOLE	BARK	WOOD	WHOLE	BARK	WOOD	WHOLE			BARK	WOOD	WHOLE			
	%	%	%	%	%	%	*gm.*	*gm.*	*gm.*	%	%	*gm.*	*gm.*	*gm.*	%	*gm.*	%
Branch roots	.....	.....	7.62	.....	.....	7.21	.....	.....	14.0	.....	3.5	.....	.....	1.07	2.5	26.9	.....
Root	17.55	2.03	6.80	9.71	3.57	5.46	19.2	43.3	62.5	30.7	15.6	3.37	0.88	4.25	9.9	111.0	37.5
Crown	17.70	5.25	10.47	7.47	3.98	5.46	17.2	24.1	41.3	41.6	10.3	3.05	1.27	4.32	10.1	72.3	46.2
1st yr. stems	16.21	6.27	11.61	8.37	3.81	6.25	54.7	47.2	101.9	53.6	25.4	8.86	2.96	11.82	27.5	175.2	57.2
2nd yr. stems	14.89	7.20	11.99	8.06	3.44	6.31	85.0	51.4	136.4	62.3	34.0	12.68	3.69	16.35	38.1	232.5	65.5
3rd yr. stems	12.84	7.63	11.16	9.94	8.56	9.50	30.6	14.8	45.4	67.2	11.3	3.93	1.13	5.06	11.8	84.2	70.0
Least sign. 5%	0.62	0.30	0.51	0.55	0.30	0.46	.....	.....	.....	1.4	.....	.....	.....	.....	3.4	.....	2.1
difference 1%	0.85	0.42	0.71	0.76	0.42	0.62	.....	.....	.....	2.0	.....	.....	.....	.....	4.6	.....	2.8
Total plant	.....	.....	10.86	.....	.....	6.46	.....	.....	401.5	.....	100	.....	.....	42.87	100	702	.....

TABLE IV

DISTRIBUTION OF RUBBER, RESIN, AND WEIGHT IN "IRRIGATED" THREE-YEAR-OLD GUAYULE
IRRIGATED IN SEPTEMBER OF THIRD YEAR (1943) AS WELL AS IN FIRST YEAR; STRAIN 130, SPACED 36 BY 24 INCHES; SAMPLED APRIL, 1944

Plant part	Rubber			Resin			Dry weight					Weight of rubber				Fresh weight	
	In dry tissue			In dry tissue			Per plant			Bark percentage	Fraction of total	Per plant			Fraction of total	Per plant	Bark percentage
	Bark	Wood	Whole	Bark	Wood	Whole	Bark	Wood	Whole			Bark	Wood	Whole			
	%	%	%	%	%	%	*gm.*	*gm.*	*gm.*	%	%	*gm.*	*gm.*	*gm.*	%	*gm.*	%
Branch roots	.....	.....	6.41	.....	.....	6.10	.....	.....	18.8	.....	4.4	.....	.....	1.21	3.1	33	.....
Root	15.45	1.62	5.68	8.70	2.96	4.64	19.9	48.2	68.1	29.2	16.0	3.08	0.78	3.87	10.0	116	36.5
Crown	16.19	4.13	9.13	6.85	3.50	4.89	19.1	26.9	46.0	41.5	10.8	3.09	1.11	4.20	10.9	81	45.9
1st yr. stems	14.29	5.31	10.20	7.82	3.73	5.94	52.2	44.2	96.4	54.2	22.7	7.46	2.35	9.83	25.5	166	57.9
2nd yr. stems	13.06	5.63	10.18	7.88	3.63	6.24	78.8	50.0	128.8	61.2	30.3	10.29	2.83	13.12	34.0	220	64.8
3rd yr. stems	11.46	5.50	9.43	9.44	7.18	8.67	44.0	22.7	66.7	66.0	15.7	5.04	1.25	6.29	16.3	125	69.8
Least sign. 5%	0.78	0.54	0.55	0.45	0.24	0.38	.....	.....	.....	1.5	.....	.....	.....	.....	4.0	.....	1.7
difference 1%	1.08	0.74	0.74	0.62	0.34	0.51	.....	.....	.....	2.0	.....	.....	.....	.....	5.5	.....	2.4
Entire plant	.....	.....	9.07	.....	.....	6.14	.....	.....	424.8	.....	100	.....	.....	38.52	100	741	.....

mid-September which brought renewed terminal growth and a crop of flowers.

Distribution through the root and stem segments (table III) is quite similar to that found in the younger plants (tables I and II). The branch roots have a higher rubber concentration than the main root, and the concentration increases from root to crown and from crown to first year stem. Not only does the second year stem have a rubber concentration comparable to the older first year stem; but the third year stem, originating entirely in the last of the three years of growth, has attained a concentration closely approaching that of the stems which have been accumulating rubber for more than two years.

Resin concentration reaches a conspicuous maximum in the most recent stem segment, the third year stem, as it did in the second year stem of the two-year-old plants. The second year stem, which presumably had a distinctively high resin content a year previously, now has the same concentration as the older first year stem. Root and crown show lower resin contents than any other parts. Stems of the above-ground branch system carry about two-thirds of the total dry weight and three-fourths of the total rubber, as they did in the case of the smaller two-year-old plants.

Within the bark portion of the plant, the root and crown show the highest rubber concentration. Above the crown, the concentration decreases with decreasing age of the stem segment. In the wood, the lowest rubber concentration occurs in the root, with an abrupt rise in the crown, and a slight rising gradient in the progressively younger stem portions. In these two tables the data for the bark and wood of the third year stem cannot be strictly compared to the corresponding tissues of the other segments by the indicated statistical standards. Samples of this stem segment were separated in only three of the six replicates. Furthermore, some selection of material was necessary to obtain stems which could practicably be separated into bark and wood; and the analyses were by the photometric, micro-method of TRAUB (**12**). However, the relative weight of dry material, rubber, and resin in the bark as compared to the wood was quite consistent, so the information from these restricted data is included for comparative purposes. According to these relative weights, the observed values for the whole stem samples were calculated into their bark and wood components to give figures comparable to those from direct analysis of the whole stems.

Resin distribution within the tissues does not show a striking pattern except for the high concentration in the root bark, the high level reached in both the bark and wood of the third year (one-year-old) stem as compared to the older stem segments, and of course the relative richness of the bark as compared to the wood.

Dry weight distribution between bark and wood displays the same relations observed in table II. In the root the bark contributes less than one-third of the dry weight. Proceeding upward in the plant to younger parts the proportion of bark progressively increases until it represents about two-

thirds of the dry weight in the case of the third year stem. The total amount of rubber deposited in the bark of the various segments comprises 71% to 79% of the total for the whole segment. Omitting the branch roots, 76% of the rubber in the plant is contained in the bark.

As a result of irrigation, the plants of table IV show a lower rubber concentration than those of table III. However, little change in the pattern of distribution can be seen. The lower level of rubber content is reflected rather uniformly through all of the parts. Resin, though perhaps at a slightly lower general level, is also very similar in distribution. Dry weight is distinctively different only in the greater weight of the third year stem. This table is offered only to show the effect of distinctive irrigation response on relative distribution within the plant, not as a typical example of irrigation culture to compare with the table III plants which were not irrigated. The terminal growth made possible by the single late irrigation was soon terminated by low temperature; and moisture differentials prevailed only until the rains of late autumn and winter.

Nine-year-old plants

Some of the relations shown in the preceding tables had already been observed in earlier samplings of the three-year-old population. It had been particularly striking that the young third year stems yielded a rubber concentration approaching that of the older stems, and that the second year stems were as high in rubber as the first year stems which were a full year older. To further investigate this tendency of young stems to attain, within one or two years, the same rubber concentration as older stems on the same plant, samples were taken from a population of nine-year-old plants (table V). With these plants it would be possible to compare a series of stem segments in which the oldest tissues ranged from one to nine years of age.

The appearance of these plants, and the segments into which they were separated are illustrated (fig. 1). Five samples of plants were separated, with two plants combined for each sample. The separation was similar to that performed on the younger plants, except that the stems were divided into biennial increments between the third and eighth year of annual elongation. Because the markings were occasionally obscure, the stem segments may not have been accurately distinguished in all cases. Nevertheless, the series from the base of the branches to the tips comprises a series of samples with decreasing average age. The planting received no irrigation until the seventh year of growth in the field, and was irrigated each summer thereafter. The relatively longer and heavier segments in the seventh and eighth year increment, which seemed anomalous before the irrigation history could be ascertained, are probably a response to the improved moisture supply.

Because of the irrigation imposed during the last three years it is impossible to draw definite conclusions concerning a tendency for equilibrium in rubber concentration through segments of varying ages. Although a gradient of decreasing rubber concentration from the first year stem to the

TABLE V

DISTRIBUTION OF RUBBER, RESIN, AND WEIGHT IN NINE-YEAR-OLD GUAYULE
NOT IRRIGATED UNTIL SEVENTH YEAR, IRRIGATED EACH YEAR THEREAFTER; STRAIN 406, SPACED 36 BY 24 INCHES IN FIELD; APRIL, 1944

Plant part	Rubber			Resin			Dry weight					Weight of rubber				Fresh weight	
	In dry tissue			In dry tissue			Per plant			Bark percentage	Fraction of total	Per plant			Fraction of total	Per plant	Bark percentage
	Bark	Wood	Whole	Bark	Wood	Whole	Bark	Wood	Whole			Bark	Wood	Whole			
	%	%	%	%	%	%	*gm.*	*gm.*	*gm.*	%	%	*gm.*	*gm.*	*gm.*	%	*gm.*	%
Branch roots	.....	.....	10.15	.....	.....	8.42	.....	.....	57.9	.....	7.6	.....	.....	5.88	5.7	102	.....
Root	20.66	3.52	8.76	9.70	5.36	6.78	34.8	79.7	114.5	30.4	15.0	7.18	2.81	10.03	9.8	187	36.2
Crown	21.82	7.77	13.27	6.97	4.55	5.30	43.1	66.7	109.8	39.2	14.4	9.40	5.18	14.57	14.2	178	43.3
1st yr. stems	21.66	9.28	15.86	8.87	4.29	6.71	61.7	53.9	115.6	53.4	15.1	13.36	5.00	18.33	17.9	187	57.1
2nd yr. stems	20.46	8.11	15.55	8.76	4.03	6.84	66.0	43.5	109.5	60.3	14.3	13.50	3.53	17.03	16.6	181	63.8
3 & 4 yr. stems	19.39	8.28	15.32	9.10	4.07	7.26	56.0	32.4	88.4	63.4	11.6	10.86	2.68	13.54	13.2	146	66.1
5 & 6 yr. stems	18.28	8.15	14.90	9.87	4.15	7.96	39.8	20.0	59.8	66.6	7.8	7.28	1.63	8.91	8.7	97	69.0
7 & 8 yr. stems	16.21	7.88	13.54	10.43	6.61	9.21	61.9	29.1	91.0	68.0	11.9	10.03	2.29	12.32	12.0	150	69.8
9th yr. stems	.....	.....	9.88	.....	.....	10.68	.....	.....	18.5	.....	2.4	.....	.....	1.83	1.8	33	.....
Least sign 5%	0.55	0.54	0.45	0.54	0.39	0.50	.....	.....	.....	1.7	.....	.....	.....	.....	.....	.....	1.6
difference 1%	0.75	0.73	0.61	0.75	0.43	0.69	.....	.....	.....	2.3	.....	.....	.....	.....	.....	.....	2.3
Entire plant	.....	.....	13.39	.....	.....	6.97	.....	.....	765.0	.....	100	.....	.....	102.49	100	1261	.....

sixth year stem is suggested, the amount of change is very slight. A noticeable decrease is seen when the seventh and eighth year segment is reached, but since all the tissues of this segment were developed in the years when irrigation prevailed, it is not known whether an effect of age or of irrigation is reflected. The magnitude of the decrease is relatively small in any case. In the ninth year (one-year-old) stem, however, a greater decrease occurs, and this segment as compared to the preceding one probably does not reflect an irrigation effect alone. Thus it appears that in an old plant a recently developed stem segment will not attain a rubber concentration comparable to the older segments until more than a year has elapsed. Within another year or two, however, it will have a concentration only slightly lower than that of stem segments in which the oldest parts are several years older.

With separation of the segments into tissues, the bark shows a maximum rubber concentration on the crown and the first year stem, with a slightly lower value in the root. From the first year stem upward, rubber concentration in the bark decreases along a gradient with decreasing age of the stem. In the wood fraction, the now familiar low concentration associated with the root occurs again. Through the other segments of wood, rubber is at a rather uniform level, except that a distinct maximum appears in the first year stem.

Resin concentration for the whole segments is at its lowest value in the crown. Above the crown a slight rising gradient is suggested, but marked increases occur only in the two youngest segments. Practically the same pattern occurs in the bark. The wood attains its highest resin content in the youngest stem segment, is at a rather uniform level in the older stems, but seems to rise progressively in the segments below and older than the second year stem.

The proportion of dry weight contained in the bark follows the same pattern as in the younger plants. The root contains the smallest proportion of bark. Upward from the root, a gradient of increasing relative bark weight extends to the youngest stems. This gradient operates to maintain a relatively uniform rubber content for the stem segments as a whole, in spite of the decreasing gradient of rubber in the separate bark and wood fractions.

Distribution of the total dry weight in the plants shows that the root comprises about the same proportion as it did in both the two- and three-year-old plants. Evidently total growth in the branches and roots occurs at approximately the same relative rates. Although the proportion of the total weight contained in the crown was similar between the two- and three-year-old plants, it appears to be higher in the nine year plants. But this is probably due to the arbitrary method of choosing the upper limit of the crown; because of the swollen development of the old crown, presumably engulfing the basal portion of the branches, more of the lower part of the branches may have been included in the crown portion of these older plants than in the younger plants.

Of the total rubber contained in the plants, about 70% is in the stems above the crown. The amount contributed by the bark ranges from 65% to 82% among the several segments. Ignoring the branch roots and the ninth year stem, which carry only a small part of the total, 76% of the rubber in the plant is confined to the bark.

Because of the high concentration of rubber and resins in bark tissues the manipulation in grinding, subsampling for extraction, and extraction frequently presents difficulties. This was particularly evident in the nine-year-old plants because of the high concentrations involved. To see if these difficulties were likely to lead to erroneous results, subsamples of entire stems were taken at the same time that the subsamples were taken for tissue separation. The rubber and resin contents determined directly on the samples of whole stem were then compared to those calculated from the analyses of separate bark and wood fractions. In thirteen such comparisons involving stems of various ages, the mean rubber content of the whole stems analyzed directly was 15.06%, and that obtained from separate analyses of bark and wood was 14.88%. A difference of 0.28% would be required for significance at the 5% level. The resin values were 7.42% when determined directly and 7.58% when calculated from separate analyses of the parts, with a difference of 0.19% required for significance. Thus it seems that, at least at this season, the composition obtained from analysis of separate tissues does not deviate conspicuously from that obtained in the commonly employed samples of whole stems or plants.

Dead parts and leaves

In the preceding tables the figures for the entire plant do not take account of the dead stems and flower peduncles or of the leaves. Therefore, to provide for a more complete description of the plants as a whole, the quantities for these parts are offered (table VI).

In the composite collection recorded as dead parts, dead shoots and the peduncles remaining from former flowers occur in varying proportions, depending on the age and history of the plant. Less than one-tenth of the dead material on the two year unirrigated plants consists of dead vegetative shoots, so the sample of dead portions is almost entirely composed of flower peduncles. On the two-year-old irrigated plants, dead shoots and twigs compose nearly a third of the total dead parts, on the three-year-old plants nearly two-thirds, and on the nine-year-old plants twigs and branches which have died back compose all but a small percentage of the weight recorded. These varying proportions of flower peduncles are largely responsible for the varying rubber and resin contents for the dead tissue sample. Only negligible amounts of rubber (about 0.3%) are to be found in the peduncles; and the resin content (between 2 and 4%), is also lower than that of dead vegetative stems.

With increasing age of plant the relative weight of dead parts evidently increases. Much of the increase is due to death of branches and twigs

resulting from competition within the plant, and perhaps to some extent from cultivation injuries. When the rubber and resin content of the whole plants (tables I–V) is corrected to include the dead parts, the resultant figures represent the values to be obtained for whole defoliated plants as they are usually taken for analysis or commercial milling. The effect of the dead parts is to lower both rubber and resin concentration only slightly in young plants, but to lower rubber more conspicuously and to raise the resin concentration as advance age increases the proportion of dead twigs and branches.

Leaves are not usually included in the weight of plant material as it is

TABLE VI

DEAD PARTS AND LEAVES
(FOR PLANTS OF TABLES I–V)

PLANT PARTS AND CONSTITUENTS	PLANT POPULATION SAMPLED				
	2 YR. I*	2 YR. IRRIG. II	3 YR. III	3 YR. IRRIG. IV	9 YR. V
Dry weight of dead stems					
Grams per plant	13.0	19.0	66.0	73.0	190.0
Per cent of entire defoliated plant	7.8	6.7	14.1	14.7	19.9
Percentage rubber					
Dead stems	0.7	1.1	2.6	2.5	5.4
Defoliated plant less dead stems	10.8	6.4	10.7	9.1	13.4
Defoliated plant plus dead stems	10.5	6.0	9.5	8.1	11.3
Percentage resin					
Dead stems	2.9	4.1	7.0	7.0	13.3
Defoliated plant less dead stems	7.1	6.1	6.1	6.2	7.0
Defoliated plant plus dead stems	6.7	5.9	6.5	6.3	8.4
Leaves, fresh weight					
Grams per plant	93.0	106.0	156.0	178.0	140.0
Percentage of entire leafy plant	30.0	20.0	20.0	22.0	9.0
Leaves, dry weight					
Grams per plant	36.0	42.0	63.0	75.0	52.0
Percentage of entire leafy plant	18.0	13.0	12.0	13.0	5.0

* Number of table in which same plant population is considered.

used for experimental studies of yields or for milling operations. The very low rubber content makes the leaves, at best, a mere dilution factor with a wide seasonal variation; and they are readily removed by a parboiling process employed by the Intercontinental Rubber Company. After immersing the plants in boiling water for a period of five to twenty minutes, the leaves can easily be shaken from the plants. However, to more completely describe the plants, the observed weights of leaves are added (table VI).

A conspicuous feature in the leaf weights appears only in the relatively small proportion of leaves on the old plants. This merely confirms a visible impression. On the tall old plants, crowded in the row, leafy shoots are confined to a horizontal plane at the top of the plant, whereas on the young plants the leafy shoots form a complete hemispherical canopy over the naked lower branches. The leaf weights recorded here are representative only of the early spring period of minimum leaf weight, just as the terminal buds

are beginning a new cycle of growth. As the leafy shoots develop in the spring and summer, the leaves of the last year cycle become senescent and wither, and by midsummer have been almost completely removed by mechanical action of wind and cultivation. In the case of the two- and three-year-old plants considered here, the total weight of leaves doubled by midsummer, and the proportional weight of leaves increased by approximately 50%. Following this maximum, representing almost entirely the leaves on the newly formed shoots, the leaf weight dropped again toward the minimum of the following early spring. The leaf samples recorded showed a mean rubber concentration of 0.3% to 0.5% and a resin content of about 10%. The rubber content is quite typical for leaves, though it may occasionally approach 1%; the resin value seems to represent a late winter maximum in a range which displays values of 6% to 8% in other seasons.

The composition of the dead branches and twigs of the nine-year-old plants offers a pertinent suggestion. Although many of the branches had grown for several years before they died, and would presumably have had fairly high rubber content, the rubber concentration of the dead sample is lower than that of any stem segment of even the two-year-old plants. Associated with this low rubber content is an extremely high resin content. This suggests the possibility that in the dead tissues rubber had been oxidized to substances which are soluble in acetone. Other interpretations are possible, however, and the possibilities were checked in the following manner. Lengths of defoliated stem were oven dried, then suspended from stakes in the field where they were exposed to the weather from October to February. The rubber content during this period dropped from 9.2% to 3.6% while the resin content rose from 7.0% to 12.1%; the differences required for significance are respectively 1.0 and 1.5 at the 1% level. Thus it seems that dead tissues in the field are very quickly subject to changes which transform the rubber into acetone soluble products. Probably sunlight is a major factor in this change, for the deleterious effects of light on rubber in vitro have long been recognized, and dried ground tissue samples held in the laboratory show little or no change over such a short period.[4] Paralleling this group of dead stems, defoliated fresh stems were exposed in the field to follow the changes associated with more natural death. However, in the damp cool weather natural drying proceeded very slowly. During the first two months the stems remained quite fresh in appearance, and after four months exposure they still contained 23% moisture as compared to an initial 40%, and to the 12% which dried stems had acquired in equilibrium with the field atmosphere. No perceptible change had occurred in the rubber content at this time, and resin content had actually decreased slightly.

One-year-old plants

Although in all the cases considered thus far the root has yielded lower concentration of rubber than the stems, this situation may not extend to very

[4] Holmes, Raiford L. Report of experiment on storing guayule samples. Unpublished report in files of Emergency Rubber Project, Salinas, California. 1945.

young plants (table VII). In this case the plants approximate one-year-old field plants in size and rubber content. However, they are the product of a direct field seeding planted in September, 1943. Low temperature brought a cessation of growth by December, so when growth resumed in the following spring the above ground portion of the plant was only a rosette. Practically all of the stem tissue and more than nine-tenths of the root tissue recorded for the February, 1945 harvest is therefore a product of the 1944 growing season. The planting had not been irrigated since July, and a final thinning at that time left a field spacing of 20 by 18 inches. The plant axis was separated into the primary root, which was cut at about six or seven inches below the soil surface, and the top portion of combined crown and stems.

In the case of these young plants the rubber concentration of the root is not significantly lower than that of the stems. Within the root, the com-

TABLE VII

DISTRIBUTION OF RUBBER, RESIN, AND WEIGHT IN ONE-YEAR-OLD GUAYULE PLANTS. STRAIN 593; SEEDED DIRECTLY IN THE FIELD SEPTEMBER, 1943; SAMPLED IN FEBRUARY, 1945

PLANT FRACTION	RUBBER IN DRY TISSUE	RESIN IN DRY TISSUE	DRY WEIGHT PER PLANT	RUBBER WEIGHT PER PLANT
	%	%	*gm.*	*gm.*
Entire plant	5.89	6.09	82.9	4.88
Stems and crown	6.04	6.50	67.3	4.06
Root	5.25	4.33	15.6	0.82
Root bark	10.86	7.15	6.8	0.74
Root wood	0.92	2.15	8.8	0.08

position of bark and wood is comparable to the two-year-old irrigated plants (table II). However, the proportion of bark in these smaller roots is greater, so the resultant rubber concentration of the whole root is higher than in the older plants.

A few samples collected from a three-year-old irrigated plot in Indio, California, provided an opportunity to check certain features of distribution which had been consistently noted in plants grown under Salinas conditions. The planting in Indio had been irrigated frequently throughout the spring, summer, and autumn, which with the high temperatures, provided for a very vigorous growth from spring until late autumn. This offered a contrast with Salinas conditions, where it seems possible that temperature limits growth even during the summer growing season. It also provided an example of the vigorous plants obtainable under irrigation in those warm areas of the southwest which seem promising for guayule culture.

Samples were taken in March, 1945, to see if variously aged stem segments show closely similar rubber concentrations, as they do in Salinas. On three-year-old plants, which were spaced 28 by 24 inches in the field, the rubber concentrations in the first, second, and third year stem segments were 7.1%, 7.1%, and 7.5%, respectively. Resin concentrations were respectively

5.2%, 5.9%, and 7.1%. These are not dissimilar from the results with Salinas plants, for rubber concentration does not differ among the segments, and resin concentration is markedly higher in the most recent stem segment.

The data from a few plants spaced more closely in the row, 28 by 12 inches, seem anomalous. Rubber percentages were 8.4, 8.4, and 6.1, respectively, for the first year, second year, and third year stem. Distinction of the third annual increment of growth, however, was not certain, and as soon as defoliated weights were available it seemed more probable that only the most recent part of the third annual increment had been taken in the part considered the third year stem. This portion of the stem displayed several increments of growth, as if cessation and renewal of growth had occurred more than once in a year's period. It is possible that the close spacing resulted in deficiencies of water during the summer in spite of the irrigation, and that some of the stem lengths selected as the third year stem contained only the more recent growth of autumn. This together with ARTSCHWAGER'S observation (**2**) that rubber does not begin to appear in cells until they are perhaps two months old or more, might explain the noticeably lower rubber concentration in the part called third year stem. But, of course, even if this were the case, the rubber concentration of the complete third year segment would probably still be lower than the other two annual segments. The comparable rubber concentrations in these two older segments, despite the difference in average age, agree with the results in other collections. Resin distribution is also on a familiar pattern, with percentages of 6.3 and 6.5 for the first and second year stems, and a distinctly higher value of 7.8% for the most recent third year stem.

LABORATORY DETERMINATION OF RUBBER COMPARED WITH MILL EXTRACTION

The question may arise as to whether laboratory determinations of rubber contents can represent the rubber obtainable in commercial extraction, for the methods employed are entirely different. While the laboratory analysis is by means of prolonged solvent extraction, commercial recovery involves mastication of the plant material under water in a pebble mill, and flotation of the resulting agglomerates of coagulated rubber particles (**10**). Table VIII is an example of a comparison between the two methods. The mill extraction was performed by the Assay Laboratory of the Emergency Rubber Project, by processes which parallel those of commercial scale extraction. Plants of variety 593, grown for three years without irrigation in a field spacing of 24 by 36 inches, were used. Four replicates, involving 40 or more plants for each type of plant material, are averaged (table VIII).

The data illustrate the fact that solvent extraction of plant tissue, as used to give the recorded rubber contents in this report, gives a reasonable estimate of the amount of pure rubber obtainable by pebble-mill procedures. When the crude rubber of the mill product is corrected for the impurities it contains, the resultant yield of pure rubber is very similar to that determined by the Spence-Caldwell solvent method. The amounts of resin and insoluble

materials admixed with the crude rubber are typical of the product from young plants; older plants usually yield somewhat purer rubber. It will be noticed that the proportional amount of resin in the crude rubber is much less than in the original plant material.

TABLE VIII

RUBBER CONTENT OF GUAYULE PLANTS BY MILL EXTRACTION AS COMPARED TO DIRECT CHEMICAL ANALYSIS OF PLANTS

EXTRACTION METHOD AND YIELD	PLANT MATERIAL	
	ENTIRE PLANT	BRANCHES ALONE
	%	%
Mill extraction		
Crude rubber	17.17	18.03
Composition of crude rubber		
Rubber	67.2	66.7
Resin	22.8	21.9
Insoluble	10.0	11.4
Pure rubber obtained by mill process	11.54	12.02
Chemical analysis of plant		
Rubber	11.97	12.41
Resin	8.70	8.92

CONTINUED RUBBER ACCUMULATION IN OLD CELLS

Comparison between the old plants of table V and the younger plants presents another intriguing question. In the root, crown, and older stems of the nine-year-old plants, the rubber concentration in both bark and wood is higher than in the corresponding parts of the younger plants, despite the fact that in the wood of the old stems, bands of dark discolored, presumably dead tissue, were frequently evident. Even in the two-year-old unirrigated plants of table I, with the highest net rubber content of any of the younger plants, the bark yielded 17.4% rubber in the root and 16.4% in the crown, with 1.7% and 5.9% as the respective values for the wood. All these are distinctly lower than the values for the nine-year-old plants of table V. This increase in rubber concentration as well as in total amount may result from two phenomena. Either rubber continues to accumulate in the original cells subsequent to the year of their formation, or the cells added in later years carry sufficiently high rubber concentrations to raise the resultant concentration of the total. Apparently there is no evidence in the literature to show whether a given group of cells will continue to accumulate rubber for several years after their initiation, or whether their rubber content remains unchanged after the first year and is therefore determined by the conditions of that year. This is a question of both theoretical interest and some practical significance. One question in cultural practices, particularly in regard to irrigation, is whether it would be possible to keep the plant in vigorous growth during the early years in the field to produce a large plant,

and then to bring more intense rubber deposition throughout the plant by withholding water.

The wood tissue, or rather the tissues inside the cambium, would seem to offer appropriate material for answering at least the theoretical question concerning the ability of some cells to accumulate rubber after their first year of existence. The tissues are arranged in order according to their sequence of formation in the plant. It follows then, that if the increase in rubber concentration for the wood cylinder as a whole is due to progressive enrichment by more recent layers of wood with high rubber content, the outer layers of wood should exhibit a much higher rubber concentration than the inner portion. On the other hand, if individual cells continue to accumulate rubber over a period of years, the inner wood and pith, corresponding to the entire wood fraction of the segment when it was younger, should yield a higher rubber concentration than is found in the wood of young plants.

A series of such comparisons is presented (table IX). The wood samples employed were from the first year stem. Thus, in the case of the material from ten-year-old plants, the pith and innermost xylem were nearly ten years old. Plants providing the material were from the same planting as the plants of table V, but since the material was taken in the following fall and winter, another year of growth had been added. The lengths of stem were selected in the field to be unbranched and without dead branches. A sample for separation consisted of one or more such lengths taken from each of three to six plants, and from three to six such samples are averaged for each comparison. In the laboratory the bark was removed and the remaining wood and pith portion cut into lengths of approximately 4 cm., which were further selected to omit any which showed remains of branches or bands of dead tissue.

To answer the question investigated, the wood cylinders could be separated into inner and outer layers at almost any intermediate point on the radius, but to provide somewhat more precise information separation was made between specific growth rings. The exposed transverse sections at the ends were polished, and the circumference of the appropriate annual ring was marked under a magnifier. The xylem outside of this ring was then peeled away with a knife to provide the sample of recently formed wood, and the remaining inner cylinder, in which the pith always appeared to be intact, constituted the sample of the early formed wood. Discernment of annual rings is subject to some uncertainty; the same factors that make the detection of annual increments of stem elongation difficult produce even more confusing patterns in the annular pattern of the xylem. However, when the total age is known it is possible to distinguish the annual rings with reasonable confidence. To give an approximation of the proportions of the wood fractions, the approximate outside radii of the several cylindrical fractions and their relative dry weights as percentages of the weight of the entire wood cylinders are included in the table.

TABLE IX

DISTRIBUTION BETWEEN OLD AND YOUNG PARTS OF WOOD CYLINDER (XYLEM AND PITH) IN GUAYULE STEMS

SOURCE OF STEMS*	COMPARISON	PORTION OF CYLINDER	ANNUAL RINGS INCLUDED	RUBBER IN DRY TISSUE	RESIN IN DRY TISSUE	OUTSIDE RADIUS OF FRACTION	DRY WEIGHT AS PERCENTAGE OF ENTIRE WOOD CYLINDER
				%	%	mm.	%
Ten-year-old plants, October	A	Inner	1–3	11.90	4.03	3.1	34
		Outer	4–10	5.13	5.74	5.7	66
		Entire	1–10	7.32	5.21	5.7	100
			L.S.D.†	0.92	0.66		
Ten-year-old plants, February	B	Inner	1–3	11.59	3.03	3.7	36
		Outer	4–10	5.88	3.65	6.7	64
		Entire	1–10	7.90	3.41	6.7	100
			L.S.D.†	0.79	0.72		
	C	Inner	1–4	10.14	3.60	3.5	48
		Outer	5–10	5.60	3.45	5.2	52
		Entire	1–10	7.78	3.52	5.2	100
			L.S.D.†	0.84	0.17		
	D	Inner	1–3	11.55	2.83	3.5	32
		Middle	4–6	5.99	3.62	5.0	30
		Outer	7–10	4.05	3.19	6.6	38
		Entire	1–10	7.13	3.19	6.6	100
			L.S.D.†	0.62	0.36		
Four-year-old plants, February	E	Inner	1–2	7.19	3.58	3.0	48
		Outer	3–4	4.71	3.41	4.3	52
		Entire	1–4	5.89	3.49	4.3	100
			L.S.D.†	0.49	0.25		

* The length of stem employed was that formed during the first year of growth; thus ten-year-old stems were used in comparisons A through D, and four-year-old stems in E.

† Least significant difference at the 5% level.

The first comparison (A) (table IX) refers to wood taken in October from the first year stem of ten-year-old plants. Rubber and resins were determined by the photometric method of TRAUB (12). The inner portion includes the pith and the layers of xylem produced during the first three years of growth. It is therefore composed of the same cells which comprised the entire wood cylinder of the stem segment when the plant was three years old. A few of the segments in the samples, however, were separated between the second and third annual rings. From the rubber values of this comparison it is obvious that increased rubber concentration in the wood as a whole with advanced age does not simply reflect an enrichment by later formed tissues with high rubber content. The wood added in the last seven years has a much lower rubber concentration than does the portion which was present at the end of the first three years. Furthermore, the 11.9% rubber now present in the part comprising the first two or three years of growth, but in which the oldest cells are nearly ten years old, greatly exceeds the concentration to be found when the oldest cells were three years old. The wood of the first year stem (tables I, II) after three years of growth contained only 6.3% rubber. The highest value for any five-plant sample included in this average was 6.8%; during a year of periodic sampling which covered the fourth year of growth without irrigation, the highest rubber content encountered in any individual sample was 7.3%. In the rubber-rich unirrigated plants of table I, samples of the first year wood taken in October after a third summer of growth to compare with the ten year plants, yielded 5.6% rubber with 7.4% as the maximum for any single sample. Therefore it seems probable that the cells of the inner part of the ten-year-old wood, those cells which were present when the wood was only two or three years old, have gained considerable amounts of rubber in the subsequent seven years.

The procedure for the second comparison (table IX, B) was similar to the first one, but the wood samples were taken four months later and the analyses were by the method of Spence and Caldwell. Again the old inner portion of the wood cylinder is not only much higher in rubber concentration (11.6%) than the layers added subsequently (5.9%), but also shows a higher value than it could reasonably have had when its oldest cells were only three years old. When the separation is made so as to include the fourth annual ring in the inner cylinder (C) the results are practically the same. However, the slightly lower rubber concentration of the inner four annual rings as compared to the inner three annual rings may be a true difference, for the net concentrations of the entire wood cylinder happen to be identical for the two comparisons. This slight reduction perhaps reflects a dilution of the three year cylinder by the fourth year wood with a lower rubber concentration. The remaining six outer annual rings of the latter comparison may also be lower in rubber than the outer seven years of the former one because of the somewhat lower average age.

Somewhat more detailed information concerning rubber distribution in the wood is presented in comparison D. To separate the cylinder into three

portions representing a gradation in average age the first three annual rings including the pith were taken as one sample, the succeeding three annual rings as another, and the most recent four annual rings as the third. Rubber concentration decreases progressively with decreasing age which indicates, if nothing more, that the new tissues added with advancing age of the segment would tend to decrease rather than increase the rubber concentration of the wood cylinder as a whole.

There is evidence in this comparison of a situation which is hinted in the previous ones. It appears that toward the innermost part of the wood cylinder rubber concentration rises more abruptly with increasing average age than it does in the outer parts. This suggests that a very high concentration may occur in the innermost tissues, presumably the pith. Unfortunately no samples of pith alone were taken. Although the pith comprises only a minute part of the wood cylinder of older stems, it is possible that the absence of a pith is one factor in the characteristic low rubber concentrations observed in the wood of roots.

The last comparison (E) is within the wood from the first year stem of four-year-old plants. Periodic samples in the same population from which these were taken showed that the rubber concentration of the wood had increased significantly since the previous winter. Such an increase, it is evident from the comparison within the wood, could not have been due to enrichment by a higher rubber concentration in the recently added wood tissue. The recent outer layers have a distinctly lower rubber concentration than does the inner part.

The rubber content in the wood of the old plants in surprisingly high, in view of Whittlesey's report that guayule wood contains no rubber. However, the differing solvent pairs involved in the Spence-Caldwell method and the method of Traub are seen to give very similar results. To further check the identity of the rubber obtained from the wood, the rubber films obtained and weighed in the Spence-Caldwell procedure were submitted to analysis by a gravimetric rubber bromide method (**14**) immediately after weighing. Eighteen such samples, including those obtained from both old and young wood samples, showed an average purity of 101.5%. Whittlesey's material may have undergone oxidation or offered some of the other difficulties which SPENCE and CALDWELL (**11**) indicate for the earlier procedures in analyzing plant material. It is possible that the wood tissues contain a less effective supply of natural antioxidants than does whole stem tissue. Stored samples of ground wood tissue seem to decrease in rubber content much more noticeably than bark or composite stem tissue. The rubber films obtained from the benzene extract of the wood samples of table IX did not show any discoloration, and gave the visible appearance of pure rubber.

The very high rubber concentrations in the old inner wood might be interpreted as merely a reflection of disappearance of other constituents in the wood. Although it would be difficult to rule out this possibility completely without following a population over several years, indirect evidence

urges that the results could not be attributed to a decrease in the reference dry weight. A drastic loss of material would be required to cause the observed increase in rubber concentration. Yet ARTSCHWAGER'S anatomical observations (1) apparently did not reveal any disintegration of the inner xylem in old plants. Furthermore, if dry material had disappeared from the old xylem it would be reflected either in a reduced ratio of dry weight to cross sectional area or in a compression of the inner cylinder. But calculation from the radii and relative dry weights recorded in table IX indicates that the dry weight/area ratio of the inner wood cylinder is not lower than that of the younger outer wood. Nor is there any evidence of compression; samples of three-year-old wood cylinders from the most vigorous branches of a three-year-old planting showed a mean diameter of 7 mm., similar to that of the inner three years in the old stems of table IX. As a further suggestion, the inner wood of comparison C (table IX) showed carbohydrate reserves at three-fourths the concentration found in the outer wood, and at about the same concentration as normally found in the wood cylinder of three-year-old plants at that season. This indicates that the reference dry weight had not been altered significantly by changes in reserve carbohydrates; and, without any sign that reserves were exhausted, it seems unlikely that extensive dissolution of more permanent xylem constituents had occurred.

Summary

Guayule plants of various ages and cultural histories are separated into several portions to display the distribution of rubber and resins in terms of both concentration and weight.

As the plant is normally harvested for experimental or commercial rubber yields, about two-thirds of the defoliated dry weight is in the branch system above ground, with only one-third in the impressively enlarged crown and root portion. A somewhat larger proportion of the total rubber is contained in the branches, for they usually carry a higher concentration of rubber than does the combined root and crown.

The tissues of the bark are the principal site of rubber deposition. Contrast between the concentration in the bark and that in the remaining wood cylinder is most striking in the root where the concentration in the bark may be eleven times that in the wood. In the branches, however, the concentration in the bark is commonly only two or three times that of the wood; for the plant as a whole the bark has approximately three times the rubber concentration of the wood. In terms of amounts of rubber the bark is even more important, because the weight of bark tissue is somewhat greater than the wood. Between three-fourths and four-fifths of the total rubber in a plant is carried in the bark.

Because rubber is more concentrated in the bark the proportion of bark in the various segments of stem and root is a factor in their resultant rubber

contents. The proportion of bark is at a minimum in the primary root; consequently, this part shows a lower over-all rubber concentration than the other parts. From the root upward the proportion of bark increases in the progressively younger parts with smaller diameters. When the branch system is separated into the successive stem lengths corresponding to successive annual increments of elongation, there is a strong tendency for the uniform rubber concentrations through stem segments of varying average ages. By the time the oldest tissues in a stem segment are one year old the segment will frequently show as high a rubber concentration as older segments on the same plant. When another year or two have elapsed the segment will be as high in rubber as earlier formed segments which are several years older. The greater proportion of rubber-rich bark in the younger stems is an important factor in this apparant equilibrium; in the bark itself there is a noticeable but still relatively slight gradient of decreasing rubber concentration from the oldest stem to the youngest. Within the wood of plants two or three years old there may be a very slight gradient of increasing rubber from the older stems to the younger ones, but in older plants the gradient disappears or may be partially reversed.

Resins or acetone soluble constituents are also concentrated in the bark. Stems which are no more than one year old show conspicuously higher resin concentrations than do older stems.

Leaves and flower peduncles yield only insignificant amounts of rubber. Dead shoots and branches are relatively low in rubber as compared to live stems. Evidence is offered to suggest that when a branch dies a considerable portion of its contained rubber is converted to acetone soluble substances within a few months of field exposure.

Evidence obtained by separation of the wood cylinder into annular parts and analyses of the variously aged components indicates that the cells of the inner xylem and pith continue to accumulate rubber for several years after they are formed. The increased rubber concentration occurring as the wood cylinder becomes older was found to be due to continued accumulation in the old inner tissues rather than to high rubber contents in the xylem added in later years. The inner annual rings of the xylem were much higher in rubber concentration than the outer ones, and higher than the corresponding annual rings of young plants.

Determinations of rubber and resin were provided by the Rubber Laboratory of this project, under the direction of Raiford L. Holmes. Dr. W. F. L. Place and Dr. J. C. Underwood, of the Bureau of Agricultural and Industrial Chemistry are to be thanked for the estimations of rubber purity by the rubber tetrabromide method.

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STUDIES IN THE METABOLISM OF CRASSULACEAN PLANTS: THE DIURNAL VARIATION IN ORGANIC ACID AND STARCH CONTENT OF *BRYOPHYLLUM CALYCINUM*

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(WITH EIGHT FIGURES)

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Crassulacean plants in general undergo diurnal variation in the acidity of the leaf tissue. They share this behavior in greater or less degree with plants of a number of other families, but crassulacean plants are the classical material for demonstration of the phenomenon; *Bryophyllum calycinum,* in particular, has been repeatedly investigated. Most studies have dealt with the titratable acidity of extracts of the leaves and with attempts to account for the chemical interconversions that take place in terms of reactions in which carbohydrates share. BENNET-CLARK (**1**) reviewed the earlier literature in 1933 and pointed out that the physico-chemical properties of polybasic organic acids must be taken into consideration in the interpretation of the results of titration. He agreed with previous workers, however, who had claimed that malic acid is the component which increases during the night and decreases during illumination of the leaves.

As a general working hypothesis to account for the observed behavior, BENNET-CLARK suggested that polysaccharides are converted to monosaccharides (sedoheptose, a 7-carbon sugar, in the case of *Sedum praealtum*) which, in turn, are oxidized to malic acid during the period when the leaves are darkened. The reverse reaction occurs during illumination. No evidence was obtained regarding the by-product of the conversion of monosaccharide to malic acid (respectively, compounds containing 6 and 4 carbon atoms) which was assumed to be a compound that contained either 1 or 2 carbon atoms (3 in the case of sedoheptose). The observations suggested, however, that carbon dioxide is not produced, and he expressed the view that, whatever the nature of the by-product, it was probably ultimately converted back into the polysaccharide. Accordingly, in its simplest form, BENNET-CLARK's speculation on the mechanisms that underlie crassulacean metabolism involves an equilibrium between malic acid and a polysaccharide; this equilibrium is upset in one direction or the other according to the conditions of illumination of the leaves.

The most comprehensive investigations of crassulacean metabolism that have been made since the appearance of BENNET-CLARK's review are those of WOLF (**14, 15, 16, 17, 18**). This investigator studied organic acid fractions isolated by extraction with ether and likewise observed a reciprocal relationship between the quantities of organic acids and carbohydrates, especially starch. He confirmed GUTHRIE's (**5**) observation that citric acid

undergoes fluctuations parallel with those of malic acid and obtained evidence for the presence of an unknown optically active organic acid which also shares to some extent in the changes.

The identification in this laboratory of isocitric acid as one of the major organic acid components of *Bryophyllum calycinum* leaves (**9, 12**), and also independently at the same time by Nordal[1] (**8**) in two other species of Crassulaceae, has solved the problem of the nature of the so-called crassulacean malic acid and throws light upon WOLF'S statements regarding the unknown optically active organic acid of Bryophyllum leaves. Moreover, the development in recent years of analytical techniques for the common plant organic acids as well as the advances that have been made in the theoretical approach to the understanding of carbohydrate metabolism in living tissues suggested that a reinvestigation of one of the oldest problems in plant biochemistry might be rewarding. A report upon the changes in the chemical composition of *Bryophyllum calycinum* over a period of 24 hours of alternate light and darkness is accordingly given in the present paper.

Preparation of samples

A group of 40 young plants that had been transplanted into sand in individual crocks on October 24, 1939 was treated with a complete culture solution containing nitrate as the source of nitrogen [see (**11**) for details of composition] for 126 days when four closely similar large plants and four similar smaller plants were selected for the experiment. On February 27, 1940, a bright sunny day which became cloudy in the middle of the afternoon, a single large and a single small plant were harvested, respectively, at 6:10 A.M. (standard time), 12:10 P.M., 4:10 P.M., and at 6:10 A.M. the following morning. The 6 to 9 fleshy simple leaves in the lowest position on the stem (hereinafter referred to as basal leaves) were collected separately, the petioles being left attached to the stem. The leaflets from the upper compound leaves formed a second and larger sample from each plant. After removal of the basal leaves and leaflets, the stem with the attached petioles was cut at the level of the sand. For each point of time, there were thus two samples of leaves and one of stem and petiole tissue for each separate plant, or 24 in all. The samples were dried and prepared for analysis as previously described (**10**).

Table I shows the numbers of basal leaves and of leaflets, the fresh weights and organic solids, the length of the stem and the organic solids of combined stem and petioles both for the four larger plants and the four smaller plants. In addition, total organic acids and starch of the leaves of the larger plants are included, the data being calculated in terms of grams per single plant. It is obvious that there was appreciable variation in size from plant to plant and that, unless a large number of plants were taken for each sample, clear conclusions could not be drawn regarding

[1] Because of war conditions, information concerning NORDAL'S observation made in 1942 was not received in this country until 1946.

changes in the chemical composition. Accordingly, it was assumed that, notwithstanding moderate differences in size of the individual plants, the *concentration* of the several analytical components would vary with time in a manner that reflected the direction and magnitude of the chemical changes that occur. Analytical data were therefore computed in terms of

TABLE I

FUNDAMENTAL DATA ON SIZE AND WEIGHTS OF *Bryophyllum calycinum* PLANTS TAKEN FOR ANALYSIS AT INTERVALS OVER A PERIOD OF 24 HOURS. FIGURES NOT OTHERWISE DESIGNATED ARE GRAMS PER SINGLE PLANT

TIME OF COLLECTION		6:10 A.M.	12:10 P.M.	4:10 P.M.	6:10 A.M.
ELAPSED TIME (HOURS)		0	6	10	24
Larger plants					
Number of leaves	Compound leaves	20	18	20	18
	Leaflets	96	74	82	78
	Basal leaves	8	6	8	9
Fresh weight	Leaflets	578.4	547.7	512.0	404.7
	Basal leaves	221.5	154.1	190.7	246.0
	Total	799.9	701.8	702.7	650.7
Organic solids	Leaflets	38.5	35.2	31.1	28.0
	Basal leaves	13.7	8.6	11.8	13.2
	Total	52.2	43.8	42.9	41.2
Total organic acids	Leaflets	16.1	11.7	9.1	11.3
	Basal leaves	4.9	2.5	3.2	4.8
	Total	21.0	14.2	12.3	16.1
Organic acids as percentage of organic solids	Whole plant	40.2	32.4	28.7	39.1
Starch	Leaflets	2.0	4.4	4.7	0.9
	Basal leaves	1.0	.....	1.3	0.8
	Total	3.0	> 4.4	6.0	1.7
Length of stem (cm.)	.....	61.5	57.0	62.0	60.0
Fresh weight	Stem and petioles	154.5	132.4	142.5	139.5
Organic solids	Stem and petioles	14.0	11.0	12.5	11.4
Organic acids	Stem and petioles	1.51	1.24	1.21	1.26
Smaller plants					
Number of leaves	Compound leaves	16	14	17	16
	Leaflets	62	43	62	59
	Basal leaves	6	8	7	7
Fresh weight	Leaflets	302.0	208.0	355.2	255.6
	Basal leaves	190.0	124.4	170.9	109.0
	Total	492.0	332.4	526.1	364.6
Length of stem (cm.)	.....	50.0	47.0	54.0	54.0
Fresh weight	Stem and petioles	114.4	87.4	117.1	107.9

1 kilogram of fresh weight of each of the three kinds of tissue examined. This implies the assumption that a kilogram of leaflet tissue collected from Bryophyllum plants in the early morning is, within reasonable limits, the equivalent of a kilogram of the same tissue collected later in the day. Because of biological variation, there can be no certainty that the successive lots of leaflets were identical in composition at the time the first sample was

collected; nevertheless, this is tacitly assumed to have been the case when the composition of such samples is compared and quantitative conclusions are drawn.

The present method of collection of samples was chosen so that the conclusions would be valid with respect to the concentration of components in the tissues of a single whole plant rather than with respect to leaves of a certain arbitrary age or size collected at random from different plants. The separate collection of the basal leaves permitted comparison of the behavior of old with that of younger tissue.

Results

The analytical data are presented as a function of time in the figures. Complete data for the leaflets of the larger plants are shown. The behavior of the smaller plants was essentially a duplicate of that of the larger plants with respect to the directions and even the slopes of many of the curves. In order to avoid unnecessary complication of the diagrams, only part of the data for the smaller plants is therefore presented. The behavior of the basal leaves also in general closely resembled that of the leaflets, but many of the changes were less extensive.

Observations on the leaflets of the larger plants (marked L) are plotted as open circles connected by full lines, those on the basal leaves (marked BL) as open circles connected by broken lines. Data for the smaller plants are plotted as filled circles connected by full lines (marked Ls) for the leaflets and broken lines (marked BLs) for the basal leaves.

Changes in acidity

The top diagram of figure 1 shows the pH of extracts of the dried leaf tissue. Independent experiments showed that determinations of pH made on juice expressed from fresh tissue were identical with those made on extracts from the same sample after it had been dried and, furthermore, that the pH of expressed juice is stable for at least 24 hours and is essentially unchanged even after the juice has been boiled. Any differences observed were at most a few units in the second place of decimals of pH and could be neglected. This result is to be anticipated because of the strong buffering action of isocitric and malic acids in the range of these observations.

The basal leaves were initially at a somewhat higher pH than the leaflets in both the large and the small plants but, in all samples, pH rose steadily during the day and fell during the night. There was a small difference in the magnitude of the change and in the slope of the curves for the basal leaves in the two sizes of plants but the curves for the two sets of leaflets were so nearly identical that only that for the large plants is shown. The magnitude of the change in the leaflets was of the order of 0.75 of a pH unit; thus the actual concentration of hydrogen ions in the plants in the early morning was about 5 times as great as that present in the afternoon. However, this does not mean that there was 5 times as much *acid*, although it indicates a substantial change.

Alkalinity of ash, shown in the middle diagram of figure 1, is expressed in milliequivalents and represents a titration of the inorganic carbonate and alkaline oxide present after all organic matter had been burned away. It is a quantity which would not be affected by changes in the organic acids unless these changes led to transport of inorganic salts into or out of the leaves. In the present case, there was little evidence of such an effect save possibly in the observation at 24 hours for the basal leaves.

Organic solids

In attempting to interpret the analytical data of the present experiment, it must be held in mind that each point of observation represents the composition of a separate plant. That the plants in each set were not exactly the same in size or weight, in spite of careful selection based on a visual appraisal of their appearance, is evident from the data in table I. Nevertheless, the plants were of the same age, had been grown under as nearly identical conditions as possible and were all members of the same clon. Irregularities less than about 10% that follow no consistent pattern in the curves are accordingly assumed to arise from such factors as biological variation or differences in hydration or both. Changes in the curves that do follow a consistent pattern and which are of a magnitude greater than 10% are assumed to have arisen from actual changes in composition brought about by the conditions of illumination. All statements must therefore be regarded as being subject to these limitations.

The curve for the organic solids of the leaves (fig. 1) furnishes a measure of the limits within which the four plants duplicate each other. The net accretion of organic solids from photosynthesis during the period of illumination was manifestly too small to be demonstrated and was certainly far less than 10% so that change from this cause can be neglected. Loss of organic solids from respiration during the night also fails to show in the curves. The four values for organic solids of the leaflets of the large plants were 66.6, 64.2, 60.8, and 69.2 gm. per kilogram. The mean is 65.2 ± 3.6, the deviation being $\pm 5.5\%$ of the mean. Thus, the organic solids were constant well within the arbitrary limitation set. The leaflets of the smaller plants and the basal leaves of both sets were even less variable. Accordingly, the actual net changes in over-all organic composition as the result of photosynthesis, respiration, or other chemical change, or in degree of hydration of the tissue were of an order no greater than about 5%.

The curve for concentration of organic solids in the leaflets of the smaller plants lies above that for the larger plants: the smaller plants evidently were somewhat less highly hydrated. The curves for the basal leaves of the two sets of plants are not shown since they were nearly identical with that for the leaflets of the larger plants. In general, then, it is evident that the composition of the leaves of the two sets was sufficiently constant to justify the assumptions made.

The curves for organic solids likewise furnish evidence of constancy of

the hydration since the water content of the samples is the difference between one kilogram and the sum of the organic solids and the ash. The curve for the ash content of the leaflets at the bottom of the figure is very nearly a horizontal straight line. Clearly, the variation in the hydration was within the limits adopted.

Nitrogenous components

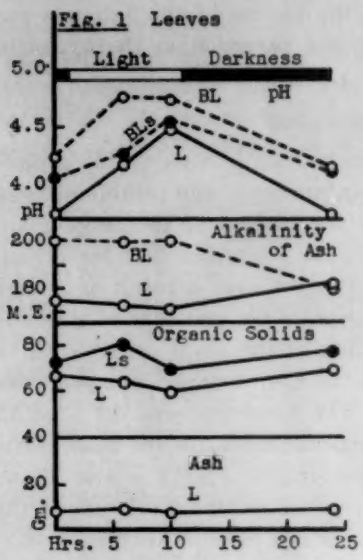
No detailed examination of the nitrogenous components of the samples was attempted but the total nitrogen, protein nitrogen, and soluble nitrogen are shown in figure 2. Total nitrogen of the leaflets of the large plants dropped slightly during the day and the curve resembles that for organic solids in figure 1. The drop might therefore have been a result of a slight increase in hydration rather than an indication of the presence of a smaller actual concentration of nitrogen in the leaflets of the plant collected in the afternoon. Total nitrogen of the basal leaves also dropped but there was no recovery during the following night. The deviation was 4.1% of the mean value for the leaflets of the larger plants and 6.3% for the basal leaves.

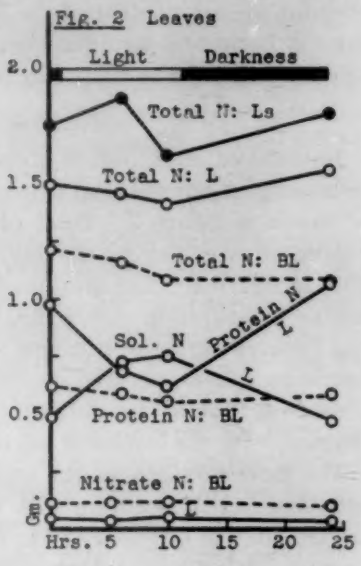
The total nitrogen of the leaflets of the smaller plants is also shown. Comparison of these curves with those for organic solids (fig. 1) shows that the variations in nitrogen and in organic solids ran parallel with each other, a further indication that the small irregularities arise chiefly from moderate differences in hydration.

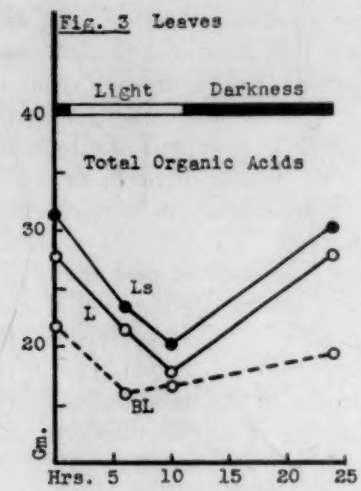
The protein nitrogen of the leaflets underwent a change that was, however, much too large to be attributed to such an effect. During the morning and early afternoon, there was a decrease that amounted to about one-third of the protein present in the early morning, but restoration occurred during the night. The curve for soluble nitrogen showed a rise during the day and a fall at night that was symmetrical both in position and magnitude with the change in protein. The increase in the soluble nitrogen amounted to about 70% of the early morning value. Similar changes were observed in the leaflets of the smaller plants (not plotted). The behavior is consistent with the view that, in the younger leaves, a substantial part of the protein underwent conversion to soluble products[2] during the day but was resynthesized during the night. No similar behavior was observed in the basal leaves of the larger plants although there was a detectable rise in the soluble nitrogen and fall in the protein nitrogen of the basal leaves of the smaller plants. That the change in the quantity of soluble nitrogen, and, accordingly, also of the protein, was not a result of the change in pH of the tissues can be inferred from these observations on the basal leaves. Furthermore, separate experiments in which the nitrogen extracted was determined after adjustment of the pH of a typical sample of dry leaf to various points within the range of the present experiment likewise showed no effect of pH on the solubility.

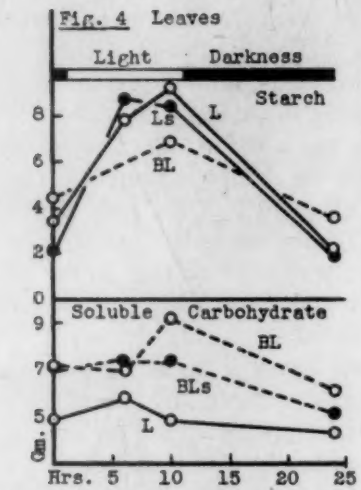
[2] That is, to products that are soluble when the dried tissue is exhaustively extracted with hot 70% alcohol and subsequently with hot water in preparation for the determination of protein nitrogen in the residue.

WOLF (**14**), who studied the nitrogen metabolism of crassulacean plants in 1931, did not encounter significant changes in the protein nitrogen of









FIGS. 1 to 4. Composition of leaves of *Bryophyllum calycinum* as influenced by light and darkness over a period of 24 hours. Data expressed in terms of 1 kilogram of fresh weight of tissue. The symbol L refers to leaflets of large plants, Ls to leaflets of small plants, BL to basal leaves of large plants, and BLs to basal leaves of small plants.

the leaves. The fact that the basal leaves did not exhibit such changes in the present experiment suggests that the material he studied may have been

too old for easily detectable alterations in the concentration of the protein to occur. He described his material as "*mittelalte Blätter.*"

Total organic acids

Figure 3 shows the changes in total organic acids, the data from both large and small plants being given. The leaflets lost about one-third of the quantity of acid present at daybreak but this had been completely restored by the following morning. The change in the basal leaves was smaller; the concentration of acids in these was lower to begin with and the loss was only from one-fifth to one-quarter of the amount present in the early morning. Furthermore, for reasons that are not readily apparent, restoration during the night was less complete. Nevertheless, the classical phenomenon of diurnal variation in acidity is clearly evident in the leaves of both sets of plants. The behavior of the basal leaves is similar to that noted by Wolf for old leaves of the same species.

Carbohydrates

Before going into the details of the changes in acid composition of these leaves, the data for starch and soluble carbohydrates (fig. 4) should be considered. Starch underwent a change in concentration in the leaflets reciprocal to that of the total organic acids reaching its maximum late in the afternoon and diminishing during the night. The magnitude of the change was less than that of the change in the acids; the concentration of starch (calculated as glucose) increased by about 6 gm. during the day, the parallel loss in organic acids being nearly 11 gm. During the night, the concentration of starch diminished by about 6.7 gm. while that of acid increased by about 10 gm. In the basal leaves, the loss of acid during the day was about 5.5 gm. and the gain of starch about 2.4 gm. as the average of the two sets of plants, but the gain in acids during the night was only about 2 gm. while the loss in starch was 3.7 gm. Clearly, the figures for loss and gain are in no case equal but they are nevertheless similar to the extent that a large change in the concentration of the acid is associated with a large change in starch.

It must be remembered, however, that the quantities mentioned are concentrations. Reference to table I, which gives quantities per plant, shows that the number of grams of starch laid down in the leaves during the day was far less than the quantity of organic acids that disappeared. On the other hand, the loss of starch during the night and the gain of organic acids were nearly equal. Thus, although it is possible that the acids synthesized during the night may have originated from the metabolism of starch, the reverse of this reaction in daylight could account for only a part of the acids that disappeared and the fate of the balance is by no means clear from the present evidence.

Total soluble carbohydrates (lower part of fig. 4), unlike starch, showed no clearly evident reciprocal relationship with acid content. Although

there was an increase during the day and a decrease at night, the quantities involved were small and the behavior of the large plants differed in detail from that of the small plants. Whether or not the small increases during the day are the result of photosynthesis cannot be demonstrated since there was no significant change in the organic solids. The small decreases at night were associated with a large decrease in starch; thus if starch was converted into a soluble carbohydrate (glucose) as its primary decomposition product, this substance did not accumulate. The velocity of the reactions whereby soluble carbohydrates were transformed into other substances must therefore have exceeded the velocity of the decomposition of the starch.

Individual organic acids

Details of the changes in concentration of the organic acids are shown in figure 5. The outstanding change occurred with malic acid; this dropped from about 16.5 gm. per kilogram of fresh tissue at daybreak to about 6.7 gm. at 4 in the afternoon but had been restored to about 15.5 gm. by the following morning. The curve for the leaflets of the larger plants only is plotted; that for the smaller plants was identical within a few tenths of a gram at all points.

Diurnal variation in acid content of crassulacean plants has from the time of Mayer been held to arise from changes in the content of malic acid but the evidence has, for the most part, consisted in determinations of the titratable acid as a measure of the magnitude together with the isolation of malic acid from enriched specimens as proof of the nature of the dominant acid.[3] The present data rest upon determinations of malic acid by a specific method and are not open to doubt regarding the identity of the acid that underwent change in concentration. Krebs and Eggleston (7) have also demonstrated diurnal variation of malic, citric, and isocitric acids in Bryophyllum leaves by specific analytical methods, and found that alterations in malic acid accounted for 83% of the total change. However, their observation was based on a single brief experiment carried out in the course of the development of an enzymatic method to determine isocitric acid.

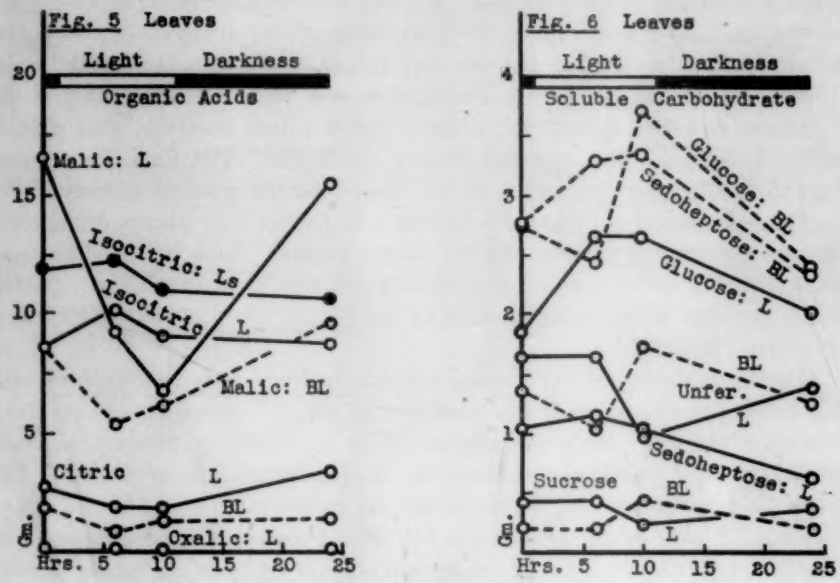
Isocitric acid increased in concentration in the leaflets of the larger plants by about 1.5 gm. during the first 6 hours, diminished slightly during the afternoon and remained constant during the night. In the smaller plants, the level of isocitric acid was higher at the start and remained constant during the morning, dropped moderately during the afternoon and remained essentially constant during the night. Isocitric acid in the younger leaf tissue therefore played a subordinate part in the changes in acidity; no clear pattern of change is manifested.

In the older basal leaves, malic acid underwent alterations that were similar to but less extensive than those in the leaflets. Only one set of data is plotted, the differences between the two sets being minor; both showed a substantial loss of malic acid during the day with recovery during the

[3] See, for example, Wolf (14) who isolated and identified malic acid as its silver salt.

night. Isocitric acid was somewhat higher in the basal leaves of the larger plants than in the leaflets at the start but diminished after 6 hours to the same point and thereafter remained nearly constant. The curve is not plotted because throughout most of its course it is indistinguishable from that for the leaflets.

Although citric acid makes up only a minor part of the total acids of Bryophyllum leaves, it shares in the progressive metabolic changes in acidity to a small extent. The data are shown in the lower part of figure 5. In the leaflets, citric acid dropped in concentration during the day by about 0.5 gm. or, roughly, one-fifth of the quantity present at dawn, and increased during the following night to a level appreciably higher than that initially



FIGS. 5 and 6. Composition of leaves of *Bryophyllum calycinum* as influenced by light and darkness over a period of 24 hours. Data expressed in terms of 1 kilogram of fresh weight of tissue. The symbols have the same significance as in figures 1 to 4.

present. The leaflets of the smaller plants behaved in the same way. In the basal leaves, the changes were smaller and were probably scarcely significant although a pattern of change similar to that in the leaflets was evident.

That citric acid, like malic acid, normally undergoes diurnal variation in Bryophyllum leaves was first demonstrated by GUTHRIE (5) although the relative importance of the change in the concentration of citric acid in the present material seems to have been rather less than it was in the leaves studied by that investigator. However, the observation has been confirmed by WOLF (17) as well as by BORGSTRÖM (2) and by KREBS and EGGLESTON (7), and the present data furnish further evidence. Nevertheless, the relative order of magnitude of the diurnal variation of citric acid seems always to be much smaller than that of malic acid.

Oxalic acid makes up a minute proportion of the total organic acids of Bryophyllum leaves and the curve for the leaflets (fig. 5) is indistinguishable from the base line. Although there appear to have been slight changes in the concentration and these followed the fall and rise in malic and citric acids, the quantities involved were far too small to suggest that this substance undergoes diurnal variation in concentration. A similar statement is true for oxalic acid in the basal leaves.

Soluble carbohydrate

Details of the changes in soluble carbohydrates are shown in figure 6 which is plotted on a scale five times that of figure 4. The data do not follow a clear-cut pattern although glucose rose slightly in the leaflets during the day and fell at night. Sedoheptose scarcely changed in concentration in the leaflets during the day but fell at night. In the basal leaves, glucose fell during the first 6 hours, then rose rather sharply during the afternoon and fell during the night. Sedoheptose, however, rose significantly during the day and fell during the night. The data for sucrose show that only minor variation of the small quantity present occurred.

The behavior of soluble carbohydrates in the smaller plants during the day was not exactly like that of the larger plants. None of the data have been plotted in order to avoid unnecessary complexity of the figure. On the whole, the only point on which most of the results agree is that there was a fall during the night.

Glucose is doubtless the monosaccharide in immediate equilibrium with starch but the velocities of the reactions in which this sugar was involved were such that only moderate alterations in the quantity present occurred notwithstanding the large changes in the concentration of starch. The metabolic relationships of the other soluble carbohydrates are by no means clear but, in view of the probable reactivity of these substances, irregularities of the type observed in the curves are all that could be expected. Truly significant results could be secured only if analyses were made of samples that represented large numbers of plants. Nevertheless, it may be noted that sedoheptose in the basal leaves behaved in essentially the manner observed by Bennet-Clark.

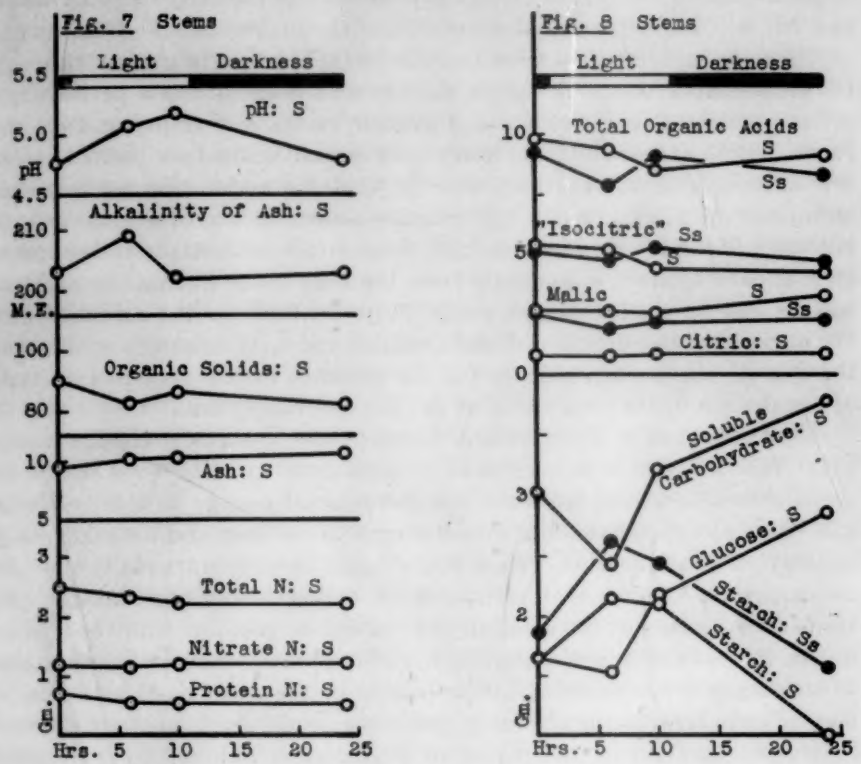
Stem tissue

Analytical data for the stem tissue are included to illustrate the contrast in behavior with that of the leaves. Figure 7 shows that the pH underwent diurnal variation but the initial point at pH 4.7 is appreciably less acid than the minimal acidity attained by the leaves in the afternoon. Not only is stem tissue considerably less strongly acid than leaves but the range through which change took place was smaller. The alkalinity of the ash was essentially constant save for the observation at noon; it is doubtful that the small increase at that time has significance.

Organic solids and ash were both satisfactorily constant for the four plants as was total nitrogen. The nitrogen content of the stem was appreci-

ably greater than that of the leaf but almost half of it was nitrate nitrogen. Of the balance, about equal parts consisted of protein nitrogen and soluble nitrogen other than nitrate. In this plant, unlike tobacco, nitrate nitrogen is apparently accumulated in the stem; only small amounts were present in the leaf (fig. 2).

Figure 8 shows the behavior of organic acids and carbohydrates. Total organic acids of the larger plants diminished slightly during the day and



FIGS. 7 and 8. Composition of stems of *Bryophyllum calycinum* as influenced by light and darkness over a period of 24 hours. Data expressed in terms of 1 kilogram of fresh weight of tissue. The symbol S refers to stems of large plants; Ss to stems of small plants.

increased to nearly the initial level at night but the relative magnitude of the change was too small to make it certain that this is an example of true diurnal variation. In the smaller plants, there was indeed a drop during the morning, but a high value for "isocitric acid"[4] in the sample collected in the afternoon suggests that increase in this component may have occurred.

[4] Although isocitric acid has recently been identified in the stem tissue of Bryophyllum in this laboratory, the component designated "isocitric acid" in this tissue is determined by subtracting the sum of the malic, citric, and oxalic acids from the total organic acids. Accordingly the presence of still other acids is not excluded. See (9) for a discussion of this point.

The data for malic acid in both large and small plants show no clear evidence of change and it may be concluded that, at least as far as the present experiment is concerned, the stem tissue of Bryophyllum plants does not undergo significant diurnal variation in malic acid. Citric acid also remained constant.

On the other hand, there were variations in the carbohydrates that were obviously significant. Starch increased markedly in the stems of both sets of plants during the day, in fact by 30% or more of the early morning value, and fell well below the initial value during the night. Much of the loss at night can be satisfactorily accounted for by the increase in glucose although the accumulation of starch during the day took place during a period when glucose remained nearly constant, a possible evidence of transport from the leaves. Both sucrose and unfermentable carbohydrate (not plotted) were low initially and changed very little so that the major changes consisted essentially of alterations in the relative concentration of starch and of glucose. There is no indication from these curves of substantial disappearance of carbohydrate components from the stem tissue during the night as was the case in the leaves and, accordingly, the data for the carbohydrates are in essential agreement with the conclusion already drawn; namely, that there is no convincing evidence for the presence of the crassulacean type of metabolism in the stem tissue of the Bryophyllum plant.

There remains to be explained, however, the systematic change in the pH. This followed a pattern entirely consistent with the view that stem tissue shares to a moderate extent in the diurnal change in acidity of the leaf tissue in spite of the fact that the acids in the stem did not change detectably in concentration. However, change in pH can equally well be accounted for in terms of alteration in the acid-base relationships. In view of the failure of the alkalinity of the ash to conform with the change in pH, this alteration could scarcely arise from changes in the relationships with inorganic components. Accordingly, in the absence of evidence, it may be assumed that the change in pH is the result of a temporary increase in organic base during the period of illumination followed by a decrease at night.

Discussion

The present data confirm and in certain details extend previous information on the diurnal behavior of *Bryophyllum calycinum*. As has long been held, variation in acidity can be largely accounted for in terms of progressive alterations in the concentration of malic acid in the leaf tissue, and citric acid shares to a moderate extent in these changes. Isocitric acid, although it is the second most important component of the leaves collected in the early morning and the predominant acid in leaves collected in the afternoon, did not, in the present experiment, undergo parallel systematic changes in concentration; it followed, rather, an irregular course.

There is every reason to assume that soluble carbohydrates are concerned in the reactions that take place, but the observed changes in concen-

tration of these components were minor ones; as has been pointed out by WOLF, it is starch which increases markedly when the acids diminish and which decreases when the acids are resynthesized. Nevertheless, on chemical grounds, it is necessary to assume that the soluble carbohydrates, especially glucose, behave as intermediates in the series of chemical reactions so that the accumulation or diminution of the soluble carbohydrates is a function of the relative velocities of these intermediate reactions. Clear demonstrations only of the accumulation or diminution of the respective end-products can be obtained.

Present day views on the metabolism of carbohydrates in living tissues, especially in the animal tissues that have been chiefly studied, suggest that carbohydrates are linked with organic acids by means of a series of enzymatic reactions most of which are reversible equilibria. Respiration in pigeon breast muscle as well as in many other tissues is specifically accounted for in terms of the Krebs tricarboxylic acid cycle and the tendency today is to invoke this fundamental scheme for the oxidation of carbohydrates in explaining many other biochemical phenomena. CHIBNALL (**4**) suggested in 1939, shortly after the scheme was originally proposed, that respiration in plant tissues may well be accounted for along similar lines, and the cycle has also served in speculative attempts to deal with some of the phenomena of amide metabolism in plants (**13**).

Crassulacean metabolism is clearly a further case upon which light may be shed by the Krebs tricarboxylic acid cycle. Even in the absence of specific information on the enzyme systems present in Bryophyllum leaves, it is obvious that the transformation of starch to organic acids at night involves oxidative reactions and there is a possibility that these reactions undergo at least partial reversal when the leaves are illuminated.

The diurnal variation in the concentration of protein in Bryophyllum leaves and the reciprocal relationship between protein and the concentration of the soluble nitrogen introduces into the general problem a new factor that may possibly be of great significance. Diurnal variation in leaf protein seems rarely to have been studied. Those investigators who have examined the nitrogenous composition of leaves throughout a single day appear to have restricted their analytical examinations for the most part to the total nitrogen. CHIBNALL (**3**), however, studied the protein content of runner bean leaves collected at nightfall and in the early morning and demonstrated that there was a fall in protein during this period of 1.8% of the evening value, and a parallel fall of 9% in non-protein nitrogen and of 2.5% in total nitrogen. These results were interpreted in terms of translocation of protein digestion products away from the leaf during the night. The change is that to be anticipated in a rapidly growing plant.

The behavior of the protein of Bryophyllum leaves appears to be something entirely different. The data, like those of CHIBNALL, are expressed in terms of concentration in the fresh weight of the tissue but they refer to the whole of the leaflets (i.e., the younger leaves) of a single plant rather

than to the relative composition of opposite members of pairs of leaflets one of which was collected at night, the other in the morning. The phenomenon is a major one; it is not a measure of the small quantity of nitrogen contributed by the leaves in elaborated form to the stem and roots in order to care for their requirements for growth. It concerns changes in the relative concentrations of protein and soluble nitrogen within the leaflets themselves during a period of 24 hours, these changes consisting of transformation into a soluble form (under the conditions of the analysis) of about one-third of the leaf protein *during the day* followed by the reverse transformation during the night. The correlation in periodicity of this phenomenon with the diurnal variation in the organic acids and the starch inevitably raises the question of a possible connection between them. Unfortunately, too little is known about the metabolism of the proteins of plant leaves in general to warrant positive statements. It seems unlikely that diurnal variation of this order of magnitude should have escaped observation hitherto if it is a common phenomenon. That this behavior should occur only in Bryophyllum is equally unlikely; it should certainly be looked for in other crassulacean plants. However, it may be pointed out that the energy requirements of the several over-all reactions are consistent with each other. Protein synthesis takes place during the night when starch decomposition and oxidation of the monosaccharides to organic acids may be supposed to supply the necessary energy; protein decomposition takes place during the period when a supply of energy is available from outside for the synthesis of starch and the transformation of the organic acids presumably in part to carbohydrates. The relationships among the numerous equilibria involved are manifestly extremely complex; that displacements in one direction or the other should occur is not surprising but attempts to assign a reason for such displacements are at present premature.

Summary

The leaves were picked from *Bryophyllum calycinum* plants successively before daybreak, at noon, and at sunset, and again the following morning before daybreak. The large simple basal leaves and the leaflets of the upper compound leaves were kept separate. The stem with attached petioles was likewise harvested. Each sample was dried at 80° C. immediately after collection and determinations were made of organic and inorganic solids, pH, protein, soluble nitrogen, nitrate nitrogen, and of the individual organic acids and carbohydrates. The data were computed in terms of concentration per kilogram of fresh tissue weighed at the time of collection.

The classical phenomena of decrease in organic acids during the day and increase at night were observed and the change was found to arise largely from alterations in the concentration of malic acid although citric acid shared to a moderate extent. Isocitric acid did not change in concentration in a similar regular progressive manner. Only a trace of oxalic acid was present and no significant change was detected.

Starch was synthesized during the day and the quantity that disappeared at night was closely similar to the increase in the quantity of organic acids. Soluble carbohydrates, especially glucose and sedoheptose, varied between moderate limits, the behavior of glucose being consistent with the view that it is an intermediate in the reactions whereby starch was presumably converted to organic acids at night.

The protein of the leaflets underwent an extensive transformation during the day to products that were determined as soluble organic nitrogen; this reaction was reversed during the night.

The behavior of the stem tissue was different from that of the leaves in that only small changes of most of the components occurred. Although there was a moderate progressive change in pH similar to that in the leaves, there was no significant change in malic acid. The concentration of starch decreased substantially at night but there was a simultaneous increase in glucose so that the net change was small. It was concluded that the stem tissue of *Bryophyllum calycinum* does not display the classical phenomena of crassulacean metabolism to a significant extent.

Although specific interpretation of the reactions is impossible in the absence of information on the enzyme systems present in the leaves of this plant, it is probable that the disappearance of starch at night coupled with the increase of malic and, to a lesser degree, of citric acid are the result of oxidation of carbohydrates by mechanisms allied to those of the Krebs tricarboxylic acid cycle. To what extent the synthesis of starch and the disappearance of organic acids during the day may represent a reversal of the reactions that occur at night is not evident from the present data.

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THE PHYSIOLOGICAL ACTION OF 2,4-DICHLOROPHENOXYACETIC ACID ON DANDELION, *TARAXACUM OFFICINALE*[1]

LOWELL W. RASMUSSEN

(WITH SIX FIGURES)

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The recent introduction of the use of synthetic growth regulating substances as herbicides is giving new stimulus to weed control activities. Because such substances are effective in low concentrations, killing plants through direct effects within the plant system and not seriously damaging the soil, they are especially desirable herbicides. The compound 2,4-dichlorophenoxyacetic acid (2,4-D) is the most widely used growth substance for weed killing purposes at the present time, and it has been shown by several workers (**6, 13, 14, 20**) to have selective action, killing most broad leaf species, while grass species are more or less resistant.

Plants which are susceptible to the action of 2,4-D show various morphological and histological responses (**7, 23, 25**) suggesting that physiological reactions of the cells are affected. Further evidence of effects on the physiological activity of the plant is indicated by the decrease in the dry weight of treated plants (**5, 16, 21**) which suggests the depletion of food reserves and possibly an increase in the respiration rate. A temporary increase followed by a decrease in the sugar content has been noted in annual morning glory [*Ipomea lacumosa* (**16**)] and in bindweed [*Convolvulus arvensis* (**12**)] as a result of 2,4-D applications. A similar response occurred in bean leaves (**17**) treated with alpha-naphthaleneacetic acid. The reserve carbohydrates of plants are rapidly depleted after treatment with 2,4-D (**16, 21, 25**) and the same effect may be caused by applications of other growth substances (**1, 15, 17, 18, 22**). Increased respiration has been shown also to follow applications of 2,4-D (**5, 21**) or indoleacetic acid (**19**), but none of these responses has been satisfactorily established as a direct cause of the death of treated plants.

The common dandelion (*Taraxacum officinale*) is readily killed by 2,4-D, and has been used in this study in an attempt to determine the physiological reactions involved in the herbicidal action of the compound.

General methods

Varying spray treatments with 2,4-D preparations were applied to heavy stands of dandelion plants in bluegrass lawns. One series of tests was run in August and September of 1945 and duplicated in June and July of 1946. Four treatments consisting of control and light (120 ppm.), medium

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(480 ppm.) and heavy (1920 ppm.) applications of 2,4-D in Weedone were made in randomized blocks with 4 or 5 replications. The light applications killed 10 to 15% of the dandelion plants; the medium and heavy treatments were about equal with kills of 90 to 100%.

Root samples were taken at the time of treatment and 5, 10, 15, 20, and 25 days afterward. A stratified method of sampling was used in which each plot was divided into four sections, each containing six sampling positions. At each sampling date roots were dug from one randomly designated position in each section within a plot and composited to make up the plot sample of at least 50 grams. Roots that were dead or that showed appreciable decomposition were not used. The samples were protected from drying and then taken to the laboratory, washed, and quickly killed in boiling 80% alcohol.

Two other blocks of plots were used; one in the summer of 1946 for additional studies of the effect of 2,4-D on root respiration, and one in the fall to compare the actions of kerosene and sodium chlorate with 2,4-D. Randomization and sampling procedures were the same as in the main experiments.

Extraction of sugars and non-protein nitrogen in the samples for chemical analyses was completed with 80% alcohol. Methods given by LOOMIS and SHULL (**12**) were followed in clearing the sugar solutions with neutral lead acetate and estimating sugars by the Munson-Walker-Bertrand method. Sucrose was hydrolyzed with invertase. Dextrin and levulins were extracted with hot water and estimated by the method given by LOOMIS and SHULL (**12**) with correction for levulose lost in dextrin hydrolysis. Starch was not present, and hemicellulose determinations were not made. Total nitrogen in the alcohol extract, by the reduced iron method, is reported as alcohol-soluble or non-protein nitrogen, and that of the residue as protein nitrogen.

Respiration measurements were made by sealing 50-gm. samples of roots in special mercury-sealed, one-liter flasks developed in this laboratory, and analyzing samples of gas from the flask after 15 to 16 hours. Volumes were corrected to s.t.p. and calculated as milliliters CO_2 produced per kilogram dry root substance per hour.

The effect of 2,4-D on the chemical composition of dandelion roots

METHODS

The changing composition of dandelion roots as influenced by 2,4-D applications at three concentrations was determined over a period of twenty-five days after treatment. None of the roots showed signs of splitting as a result of any increased growth which might have occurred. The root populations of the plots under the medium and heavy treatments were noticeably reduced because many plants were completely killed by the time of the last two samplings. Consequently, the samples on which composition studies were made consisted only of those roots which had resisted the action of the

treatment enough to be still present. When the twenty- and twenty-five-day samples were taken, most of the top growth appeared dead under the medium treatment and only a slight trace of life in the tops was found under the heavy treatment, however, some of these plants which appeared to be dead, even down into the crown, still had roots which appeared normal after the crown was removed.

The results of the two main experiments, run in 1945 and 1946, were very similar; we have, therefore, conserved space by showing data only for the latter season.

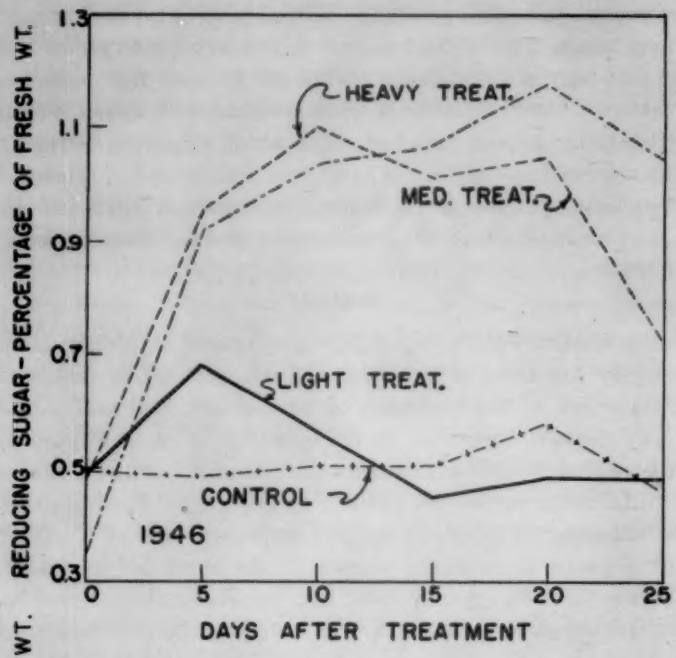
Results

Reducing sugars.—Reducing sugars are formed by plants during photosynthesis or by digestion of reserves, and are utilized in respiration or as building materials in the synthesis of protoplasm, cell walls, and storage forms. Any change, therefore, in the quantity of these sugars present as a result of 2,4-D application indicates the possible reactions that are being affected. The reducing sugars present in 100 gm. of fresh dandelion roots from the 1946 tests are shown in figure 1 as percentages of the fresh weight.

The percentage of reducing sugars in the roots before treatment was slightly higher in 1945 than in 1946, and the reductions shown in figure 1 at 20 days started at 10 or 15 days. In both seasons treatments with 2,4-D caused an early rapid increase in reducing sugars followed by a decrease. A large increase in the reducing sugar content of roots suggests an accelerated hydrolysis of the polysaccharide reserves. The later decline might result either from a slowing down of the hydrolytic action or an exhaustion of the reserve carbohydrates.

Sucrose.—The sucrose content of the 1946 roots and the changes resulting from the treatments are plotted as percentages of the fresh weights in figure 2. The initial sucrose content of the roots was about 1% higher in the 1946 samples taken in early summer than in the late summer samples of 1945. In both tests the 2,4-D applications caused a slight decrease in sucrose while the controls were showing an increase.

Polysaccharides.—The polysaccharide reserves of dandelion roots are mainly a mixture of dextrins and levulins, as shown by Loomis (11) and verified by tests of our material. Dextrins constituted 2 to 3% and levulins 6 to 10% of the fresh weight of the freshly dug, untreated roots; both fractions being somewhat higher in 1946 than in 1945. Dextrin percentages dropped to about half of the control values with medium and heavy 2,4-D treatments. Percentage losses of levulins were comparable, with total losses correspondingly greater. The 1946 data for levulins are plotted in figure 3. The light treatment, which killed only a small percentage of the plants, gave the smallest levulin and dextrin losses, but considerable quantities of polysaccharide reserves were left in the heavier treatments which gave 90 to 100% kills. Disintegration of the dead roots was so rapid that it was not possible to obtain samples at what might be considered the death point.



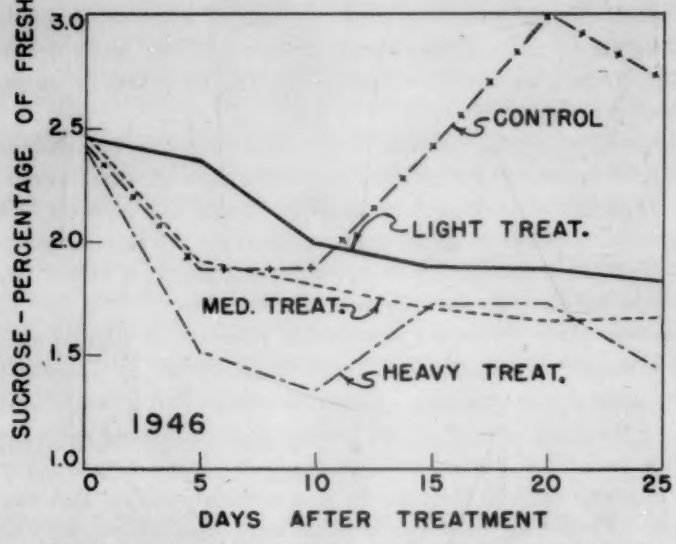


FIG. 1. (Above) Percentages of reducing sugars in control and treated dandelion roots—fresh weight basis.

FIG. 2. (Below) Percentages of sucrose in control and treated dandelion roots—fresh weight basis.

Plants were dying rapidly in 15 days, however, and by 25 days it was difficult to find enough roots for samples. It seems to be a fair assumption, therefore, that the roots died before their reserves were exhausted. This conclusion is supported by the data for total reserves (sucrose, dextrins, and levulins) shown in figure 4.

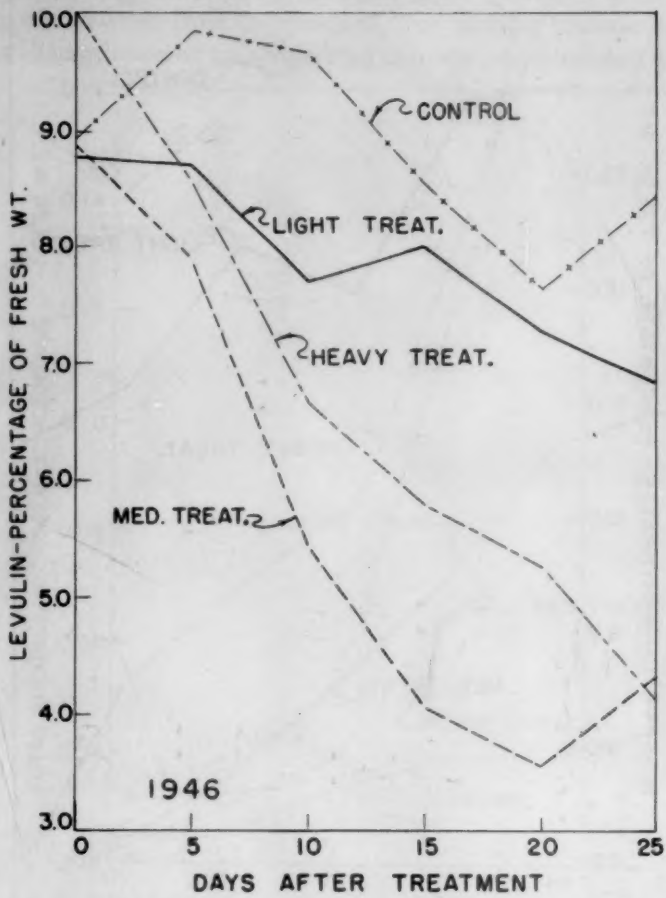


FIG. 3. Percentages of levulin in control and treated dandelion roots—fresh weight basis.

The decrease in reserve carbohydrates was very much more than enough to account for the increase in hexose sugars, which suggests that a large amount of the sugars resulting from the hydrolysis of reserve foods was either used up in respiration or in the synthesis of non-carbohydrate materials or both.

NITROGEN ANALYSES.—The nitrogen content of plant parts gives an indication of the physiological reactions that are taking place. A high soluble nitrogen content indicates synthesis or hydrolysis and may stimulate new

growth. The protein-nitrogen content indicates whether protoplasm is being synthesized or destroyed.

Nitrogen soluble in 80% alcohol (non-protein N) was less than 0.03%

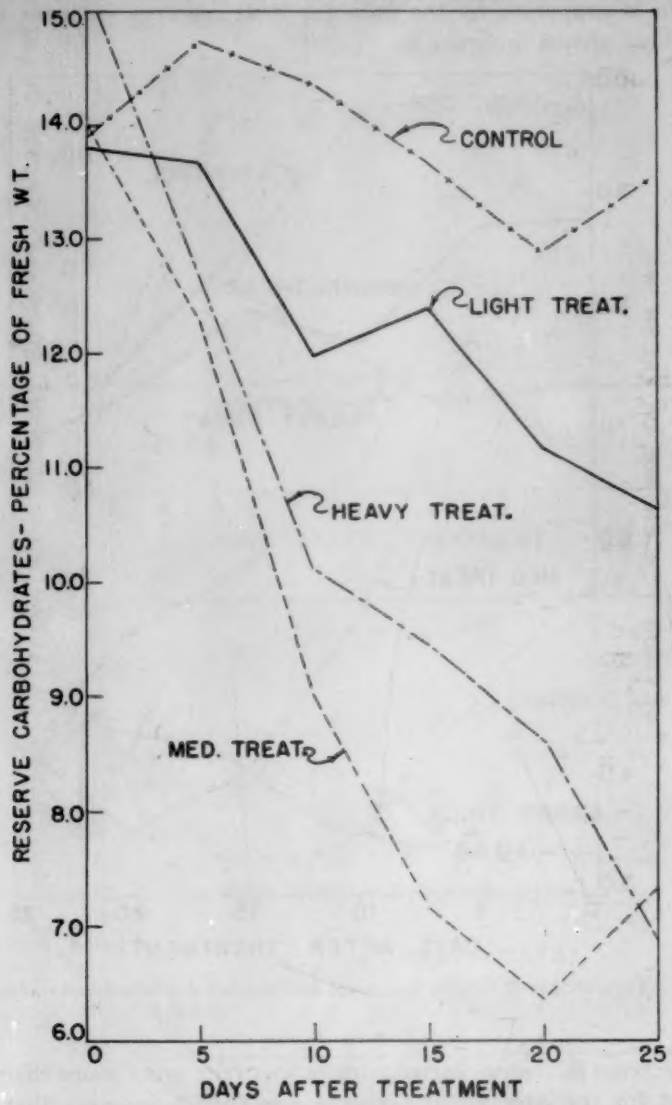


FIG. 4. Percentage reserve carbohydrates in control and treated dandelion roots—fresh weight basis.

in June of 1946 and up as high as 0.08% in August of 1945, with an upward trend in the controls of both series. The effect of 2,4-D treatments (fig. 5) was to raise these percentages by 2 to 5 times, with the heavy treatment tending to show the least effect and the light treatment the most. If soluble

nitrogen is considered to be stimulating for cell division (10), these results suggest that the lighter treatments should have been more stimulating, while the heavier were showing direct toxicity.

The protein nitrogen also showed a tendency to increase during the period of each test, although the light and heavy treaments of 2,4-D did not cause much deviation from the controls. The medium treatment caused the greatest change, showing an accelerated increase which reached a maximum

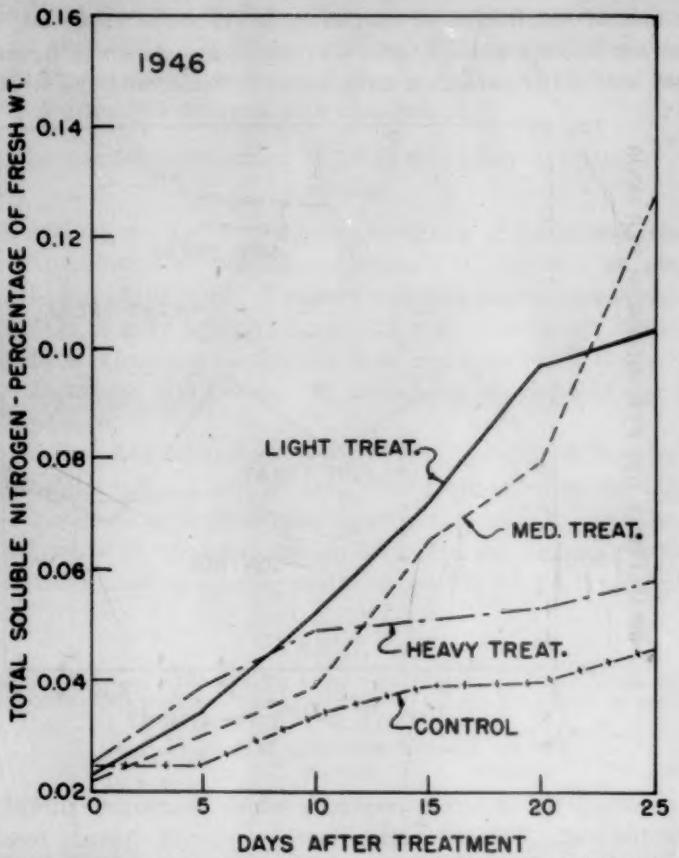


FIG. 5. Percentage total soluble nitrogen in control and treated dandelion roots—fresh weight basis.

fifteen days after treatment. The increase in both protein nitrogen and soluble nitrogen suggests an increased rate of absorption of nitrogen compounds from the soil and the synthesis of amino acids and proteins. It is possible that the increased protein nitrogen was merely an indication of the depletion of the carbohydrate reserves, but the expression of the results on a fresh weight basis would reduce this error, and the small increases in the heavy treatment, which gave the largest carbohydrate losses, suggest that it was not a factor.

The effect of 2,4-D on the respiration of dandelion roots

The rate of respiration in plant tissue is a measure of reaction intensity, and it is affected by both internal and external factors. The application of toxic substances to plants often stimulates an increase, usually followed by a decrease, in respiration while the normal response to a small quantity of growth substance is an increase in respiration which continues at a high rate as long as the reactions are possible (**4, 24**).

The rate of respiration of root samples from the 1946 test, on which chemical composition studies were also made, are shown in figure 6. The treatment with 2,4-D caused an early, rapid increase, with the medium treat-

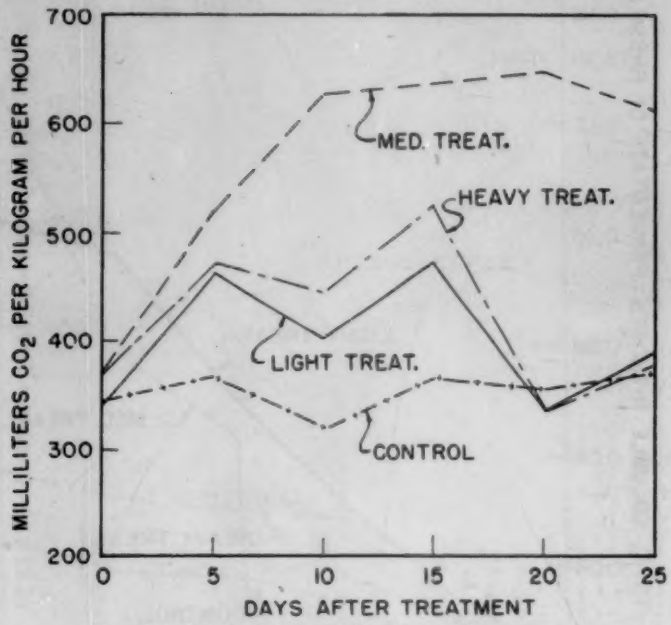


FIG. 6. Rates of respiration in dandelion roots.

ment producing the greatest response, which continued throughout the period of the test. The percentage increases for the plants receiving the medium treatment were 92, 75, 83, and 66 at 10, 15, 20, and 25 days, respectively, after treatment. This increased respiration rate with the medium treatment was concurrent with the increased reducing sugar content (fig. 1), and suggests the possibility of the high sugar content causing the accelerated rate of respiration. The heavy treatment of 2,4-D, however, indicates that the effects on sugars and respiration are two separate processes, as this treatment increased the sugar content considerably throughout the test periods, but increased respiration only slightly for the first 15 days, after which it decreased, even though the sugar content was high.

The results between 5 and 15 days would fit well an hypothesis of a toxic action of 2,4-D, with an increasing stimulation of respiration from light to

medium treatments and a drop with the excess, heavy treatment. The 20-day lows for light and heavy applications would then represent, respectively, the wearing off of the light effect and progressive destruction of protoplasm and enzymes with the heavy treatment. If this hypothesis is accepted it should be noted that the stimulating effect of the medium treatment on respiration continued until near the death of the roots. A second series of experiments with the medium treatment only showed this same effect; respiration was doubled within 10 days after treatment and remained high until the death of the plants. If these reactions are indicators of direct protoplasmic toxicity, as we believe they are, they indicate an action on some part of the cell not directly concerned with respiration.

The comparative action of 2,4-D and other herbicides

Methods

An experiment was run to compare the effects of 2,4-D, 960 ppm., with kerosene, 200 gal. an acre and sodium chlorate, 475 pounds an acre, when applied in the fall of the year. Kerosene was used because it has been shown by Loomis (**11**) to have selective herbicidal action in killing dandelions in lawns. Sodium chlorate is a commonly used herbicide which has been shown by Bakke, Gaessler, and Loomis (**2**) to cause a depletion of the food reserves in bindweed.

The chemical determinations reported for the fall experiments were made on samples taken 4 and 14 days after application of the treatments. The data for the two determinations are shown in table I. The data of the 14-day analyses were analyzed statistically, and the mean significant difference was calculated for comparing the values for the 2,4-D treatment with the others.

TABLE I

Carbohydrate analyses of dandelion roots taken 4 and 14 days after treatment. Means of three replicates expressed as milligrams per 100 grams of fresh roots

Treatment	Reducing sugar		Sucrose		Dextrin		Levulin	
	4 days	14 days*	4 days	14 days†	4 days	14 days‡	4 days	14 days§
Control	302	140	2733	2688	2060	2598	6833	7916
Kerosene	970	1467	1743	2432	1888	1305	6432	3676
Sodium chlorate	152	457	1382	1767	2225	1912	7991	5834
2,4-D	323	410	1621	1662	1768	1270	5885	3564

* Significant mean difference from 2,4-D 410 ± 286.
† Significant mean difference from 2,4-D 1662 ± 532.
‡ Significant mean difference from 2,4-D 1270 ± 571.
§ Significant mean difference from 2,4-D 3564 ± 1759.

Results

Carbohydrates.—Kerosene caused an increase in reducing sugar to a percentage significantly higher than that for 2,4-D. The 2,4-D treatment in

this fall test did not cause a significant increase in reducing sugar over the untreated controls, differing in this respect from the large increases caused in the summer tests.

At 4 days after treatment the sucrose content was significantly lower in the roots from treated plots compared with control, but there was no difference among the three herbicides. At 14 days, however, 2,4-D and sodium chlorate-treated roots were still lower in sucrose than the control, but the sucrose had increased under the kerosene treatment to a level significantly higher than the other two herbicides, and equal to the control (table I).

Four days after treatment there were no significant effects on the dextrin content of the roots. At 14 days the dextrin content in the roots from plots treated with the herbicides was significantly lower than in the controls. The dextrin content of the 2,4-D-treated roots was lower than the sodium chlorate-treated roots; kerosene and 2,4-D caused comparable changes in the dextrin content.

TABLE II

NITROGEN ANALYSES OF DANDELION ROOTS TAKEN 4 AND 14 DAYS AFTER TREATMENT. DATA ARE MILLIGRAMS PER 100 GRAMS OF FRESH ROOTS

TREATMENT	SOLUBLE NITROGEN		PROTEIN NITROGEN	
	4 DAYS	14 DAYS*	4 DAYS	14 DAYS†
Control	171	207	171	173
Kerosene	143	189	196	222
Sodium chlorate	216	197	193	200
2,4-D	134	100	184	198

* Significant mean difference from 2,4-D 100 ± 68.
† Significant mean difference from 2,4-D 198 ± 11.

There were no significant differences in the levulin content four days after treatment although the 2,4-D treatment showed a lower quantity than the other treatments. The levulin content decreased for each of the herbicidal treatments to a level significantly below the control by the fourteenth day. There was no difference in the effect of 2,4-D and kerosene on the levulin content, but the sodium chlorate treatment was significantly higher.

NITROGEN.—The total soluble nitrogen content of the roots and the effect of the treatments on this component are shown in table II. The differences four days after treatment were not significant, as indicated by an analysis of variance. However, by 14 days after treatment the quantity of soluble nitrogen in the roots from plots treated with 2,4-D had decreased to a level significantly below all of the other treatments. This response is different from the effect produced by the lighter applications in the summer tests in which the soluble nitrogen increased in the roots.

The herbicidal treatments caused an increase in the protein nitrogen of the roots, as shown also in table II. The differences, however, were small and were not significant until 14 days after treatment. The 2,4-D and

sodium chlorate gave the same response, while kerosene caused an increase significantly higher than 2,4-D.

RESPIRATION RATES.—When root samples were taken for chemical analysis, enough extra roots were dug to make respiration rate measurements on each treatment with two replicates. The mean rates of respiration and the respiratory quotient for each treatment are given in table III. Both 2,4-D and kerosene caused large increases in the rate of respiration, but the type of materials being utilized or some steps in the process were evidently different, as indicated by the higher respiratory quotient for the kerosene treatment.

In this test the 2,4-D treatment caused a large increase in respiration, but did not cause an increase in reducing sugars (table I), which indicates again that the respiration rate of the 2,4-D treated roots was not correlated with sugar content.

TABLE III

RESPIRATION RATES EXPRESSED IN MILLILITERS CO_2 PER KILOGRAM OF DRY ROOT MATERIAL PER HOUR, AND THE RESPIRATORY QUOTIENTS OF DANDELION ROOTS—1946

TREATMENT	DAYS AFTER TREATMENT		RESPIRATORY QUOTIENT
	4	14	
Control	405	408	0.94
Kerosene	934	869	1.24
Sodium chlorate	462	645	0.96
2,4-D	682	1059	0.97

Discussion

The reducing sugar content of the roots from treated plants was increased but the sucrose content was decreased more than enough to account for the rise in reducing sugar, the net effect being a decrease in total sugars. The reserve polysaccharides, dextrin and levulin, decreased rapidly after the 2,4-D treatment. These changes in the carbohydrates of the roots are in agreement with those reported by SMITH, HAMNER, and CARLSON (**21**) for bindweed roots, and MITCHELL and BROWN (**16**) for annual morning glory. Other growth substances produce similar effects according to several investigators (**1, 15, 17, 18, 22**). The increase in reducing sugars is probably the result of an accelerated hydrolysis of reserve carbohydrates. Depletion of the total carbohydrate reserves was marked, but it is doubtful that the plants were killed by starvation, since the greatest decreases amounted to only 68% of the total in 1945 and 49% in 1946. The carbohydrate losses caused by the three concentrations of 2,4-D after 25 days are summarized in table IV. The milligrams of total carbohydrates lost as a result of each treatment concentration were about the same each year even though the percentage decrease was greatest in 1945. It is interesting to note that increasing the concentration of 2,4-D four times (120 to 480 ppm.) caused a twofold decrease in carbohydrates, while a further concentration increase of four times

(480 to 1920 ppm.) caused only a slight additional decrease, showing that the lower concentrations of 2,4-D gave the greatest physiological response in relation to the quantity applied.

The decrease in carbohydrate content of the roots is reflected in a decrease in the total dry weight of the roots, and a comparison of these decreases gives an indication of the reactions involved. A decrease in dry weight equal to the decrease in carbohydrates suggests utilization of the carbohydrates in respiration, while a decrease in dry weight less than the decrease in carbohydrates would indicate some synthesis of non-carbohydrate materials. We found in our experiments that the decrease in dry weight was practically equivalent to the loss in carbohydrates, indicating that very little if any carbohydrate was converted into non-carbohydrate

TABLE IV

DECREASE OF RESERVE CARBOHYDRATES CAUSED BY 2,4-D, 25 DAYS AFTER TREATMENT

TREATMENT—2,4-D	SUCROSE		DEXTRIN		LEVULIN		TOTAL	
	1945							
	mg.	%	*mg.*	%	*mg.*	%	*mg.*	%
Light	983	36	310	19	1586	29	2880	29
Medium	1446	54	691	42	3314	61	5452	56
Heavy	1857	69	879	53	3942	73	6679	68
	1946							
Light	879	32	430	18	1613	19	2921	22
Medium	1062	39	1082	45	4098	49	6241	46
Heavy	1284	47	1063	44	4331	51	6677	49

material. Consequently little or no growth or differentiation should be shown, and in confirmation there were no indications of growth in the dandelion roots at any time during the tests. In this respect the response of dandelion roots is different from that of bindweed and sow thistle roots reported by TUKEY et al. (**25**) who observed large increases in root and rhizome diameters accompanied by splitting. They also found that starch disappearance was correlated with new cell growth. It is probable that detailed histological studies of the dandelion root would reveal at least some growth or differentiation, since such a response in other plants has been shown by several investigators (**3, 8, 9, 23**). Apparently, however, the growth responses are small in the dandelion roots.

The 2,4-D applications approximately doubled the respiration rates of the dandelion roots. The heavy application, which was the most rapidly toxic to the plants, caused only a temporary increase in respiration which

dropped back to normal or below after 15 days. The medium treatment, which was sufficiently toxic to kill most of the dandelions, caused a large increase in respiration which remained high throughout the test, showing only a slight drop after 20 days.

Since the decrease in carbohydrates was apparently all due to oxidation, the question was raised as to whether the increased rate of respiration was sufficient to account for the carbohydrate loss. To accurately determine the balance between the rate of respiration and carbohydrate loss would require information on the rate of photosynthesis, and such data were not obtained. The data we have, however, give some indication of the balance. The mean daily loss of carbohydrates was determined and the rate of respiration necessary to account for the loss was calculated. It was estimated that if the light and medium treatments caused a decrease in photosynthesis of one third, then the observed rate of respiration would account for the loss of carbohydrate. The heavy application would have had to practically stop photosynthesis in order for the observed rate of respiration to account for the carbohydrate loss. Since the leaves were quickly killed by this spray this assumption is not unreasonable.

A study of the effect of 2,4-D, kerosene, and sodium chlorate on the composition and respiration rate of dandelion roots showed that 2,4-D and sodium chlorate had the same effect on reducing sugars, while kerosene caused a much greater increase in reducing sugars and only a slight decrease in sucrose. Kerosene and 2,4-D caused similar decreases in dextrin and levulin, while sodium chlorate was less effective. A check on May 20, 1947 of the plots of the 1946 fall test showed nearly a complete eradication of the dandelions in the 2,4-D plots, and practically equal kills with kerosene, while the sodium chlorate treatment was the least effective. The grass was nearly all killed by the chlorate, slightly injured by the kerosene, but not visibly injured with 2,4-D.

The striking effects of the differential herbicides, 2,4-D and kerosene, were increased digestion and respiration of reserve carbohydrates. The plants did not, as early popular accounts have suggested, "grow themselves to death"; neither did they starve to death, and the respiration should in our opinion be classed as a symptom of specific toxic effects rather than the direct cause of injury. The resistance of the grasses and of some other plants to these differential sprays suggests that the toxicity of these substances may be directed toward certain cytoplasmic compounds, probably proteins, which vary between families and to a lesser extent between genera or species.

Summary

1. The reducing sugar content of dandelion roots increased rapidly following applications of 2,4-dichlorophenoxyacetic acid, but later decreased towards the level of the control. The sucrose of the roots declined slowly after treatment, and the dextrin and levulin contents decreased rapidly.

The decrease of carbohydrate reserves was considerably more than enough to account for the increase in reducing sugar. It was found that the loss could be roughly accounted for by the increased rate of respiration, indicating little if any utilization of sugars for growth response.

2. Respiration rates increased with the 2,4-D; the low concentration giving only a temporary stimulus with a return to normal, possibly because of the small quantity of 2,4-D, while the medium concentration gave the greatest increase which remained high as long as the roots were alive. The highest concentration caused a temporary increase followed by a decrease.

3. A comparative test of 2,4-D with kerosene and sodium chlorate showed that 2,4-D and kerosene were equally effective in decreasing the carbohydrate reserves and in increasing the rate of respiration, of dandelion roots, while sodium chlorate was less effective. Kerosene and 2,4-D were approximately equal in eradicating the dandelions with little or no effect on the grass, while sodium chlorate gave slightly less kill of dandelions and killed nearly all of the grass.

4. From the results of the studies it appears that the action on dandelion of 2,4-D in herbicidal concentrations is principally the destruction of carbohydrate reserves, with most of the loss being accounted for by increased respiration. It seems improbable that these effects alone can account for the lethal action of 2,4-D and we have postulated that they are symptoms of direct, specific, protoplasmic toxicity rather than causes of injury.

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GROWTH OF THE DANDELION SCAPE[1]

MARIAN DELLERS CHAO

(WITH SIX FIGURES)

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The growth of the flowering stalk of dandelion, *Taraxacum officinale*, is of considerable interest because of the presence of two distinct periods of rapid growth. The last of these precedes and accompanies the maturation of the fruit, and represents a resumption of rapid growth in what would normally be considered a mature organ.

MIYAKE (**8, 9**) showed that growth of the dandelion scape may be divided into three phases: the first a fairly rapid, uniform growth, the second a phase of slow growth; and the third, a rapid growth in the upper portions of the organ. SCHMALFUSZ (**10**) showed that mitotic figures were present in elongating scapes up to the stage of shedding ripe fruits and were especially frequent preceding the periods of greatest scape elongation.

As a part of a general study of the mechanism of cell elongation the experiments of MIYAKE and SCHMALFUSZ have been repeated and extended. The data presented were obtained from extensive studies of the regions and periods of cell division and elongation in the dandelion scape, together with studies of cell volume, green and dry weight, and nitrogen content on a per cell basis.

Cell division and enlargement

GROWTH OF SCAPE

A uniform plot of uncut sod with a large proportion of dandelions was used for these studies. The dandelion scapes grew irregularly over a period of 32 days. In the first phase, which occupied about nine days in the material tested and lasted from the first appearance of the scape until the blossom opened, the scapes grew rapidly and uniformly throughout their length. The second phase, a period of slow scape growth, began just before the blossom closed and extended over a period of about twelve days, corresponding to the time of embryo development. The final phase covered an eleven-day period beginning sometime before the opening of the seed head and continuing for several days after all the seeds were dispersed. During this time the scapes again grew vigorously.

The growth curve for the whole scape, based on the average of values for forty typical scapes, is shown (fig. 1a). Whereas the usual growth curve of an organ is S-shaped, this curve has two S-shaped regions with a flat region of slow growth interposed. For comparison the growth curves of the

[1] A portion of a thesis presented to the Graduate Faculty of the Iowa State College in partial fulfillment of the requirements for the degree Doctor of Philosophy. A complete copy of the thesis is on file at the College Library.

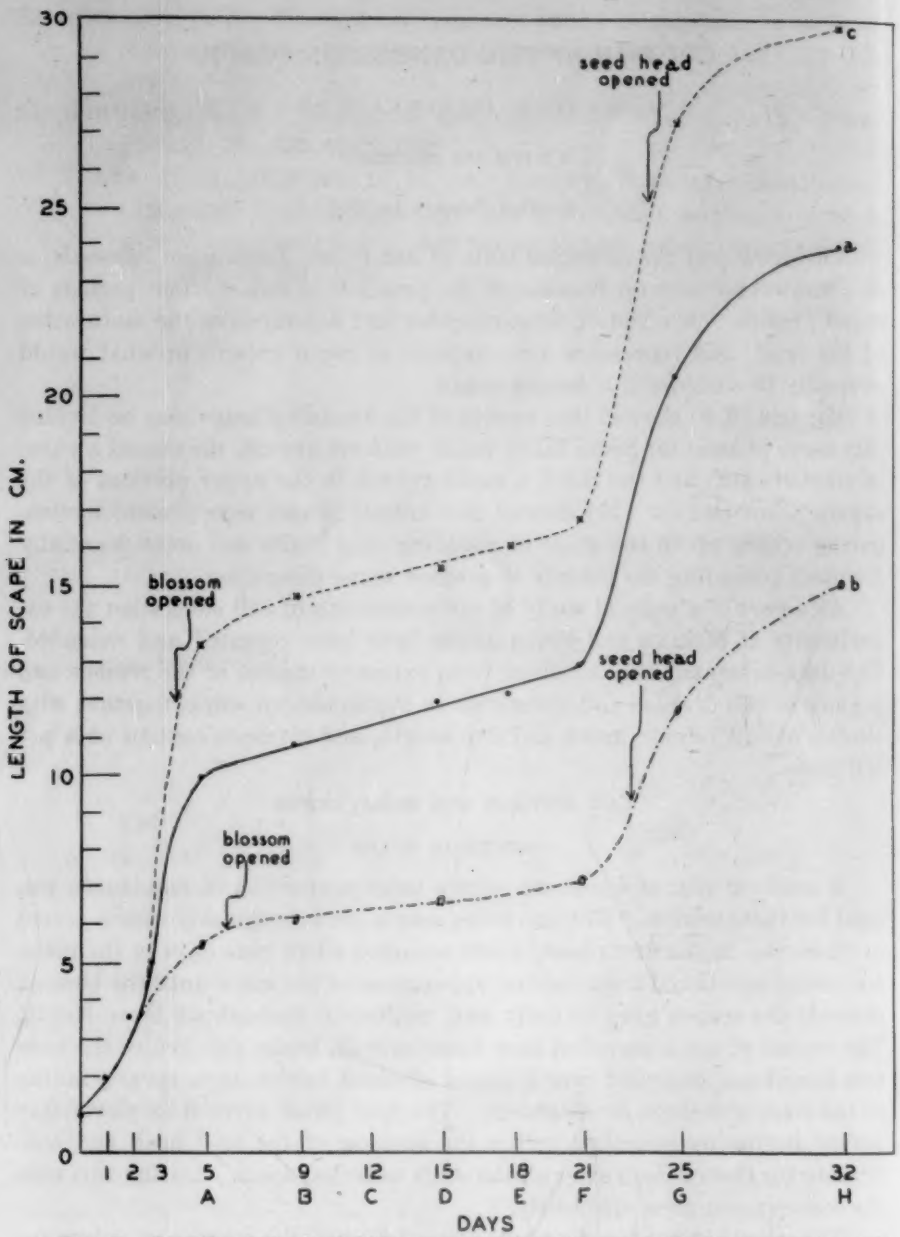

FIG. 1. Growth of the dandelion scape. a. Average scape; b. short scape; c. tall scape.

single scape attaining the smallest final length (fig. 1b) and the single scape attaining the greatest final length (fig. 1c) are also shown. The shape of these three curves is similar and the three phases can be distinguished in each.

Cell growth and section growth

Bud-scapes averaging 7.26 cm. in length were marked with India ink at 1-cm. intervals for the main growth experiment. Eight times during the period of development the lengths of the sections between markings were recorded. The average cell length in stripped epidermal sections from parallel material, mounted in water, was measured with an eyepiece micrometer at the same times. Cell length at each stage was determined for sections of the scape immediately under the floral parts (top), center-scape sections (center), and sections at the base of the scape (base). For each section at a given stage, twenty epidermal cell lengths were measured from each of four scapes and the average of these eighty cells used for estimations of changes in cell size and number. In parallel experiments bud-scapes as small as 1 cm. in length were marked with India ink to observe zonal growth, and epidermal sections were measured for cell length.

Data from these experiments are summarized (table I, fig. 2). In the earliest stages, before the bud-scapes were 5 cm. long, cell division was rapid in the upper portions of the scape as shown by the small or negative increase in cell lengths and the doubling in length of the sections. Basal sections showed less cell division; however, the small increase in cell length relative to section length at the 5-cm. stage is interpreted as cell division. At stage "A," blossom open, all three zones grew rapidly and primarily by cell elongation. After the blossom head closed, the scape grew very slowly. The elongated cells in the sections at the top of the scape underwent an average of one division each, as the average cell length decreased by about half and the scape as a whole showed little elongation. In the middle of the scape cell divisions also occurred, but probably only about half of the cells (the longer ones) divided, and no more than one division occurred in any one cell. At the base of the scape there was even less activity, and probably only scattered cell divisions occurred in the longer cells, as the average epidermal cell length decreased only slowly and to a small extent.

During the last rapid growth of the scape, cell elongation again kept pace with scape elongation, indicating that few or no cell divisions occurred at this time. The most rapid growth took place at the upper end of the scape. The center of the scape showed less growth, and the base showed only a small amount of elongation.

Camera lucida drawings of epidermal cells from the center of typical dandelion scapes at the various stages, show the sequence of events in the cells during scape growth (fig. 3). The small cells seen in the bud-scape (fig. 3A, 3B) elongated until they attained a great length at the open blossom stage (fig. 3C). After the blossom closed, these extremely long cells began to divide (fig. 3D) and the new cell walls (shown as stippled) were easily distinguishable from the older walls, being less distinct in appearance. Before the seed head opened none of the very long cells was visible, cell divisions having produced two shorter cells for each long one (fig. 3E). As

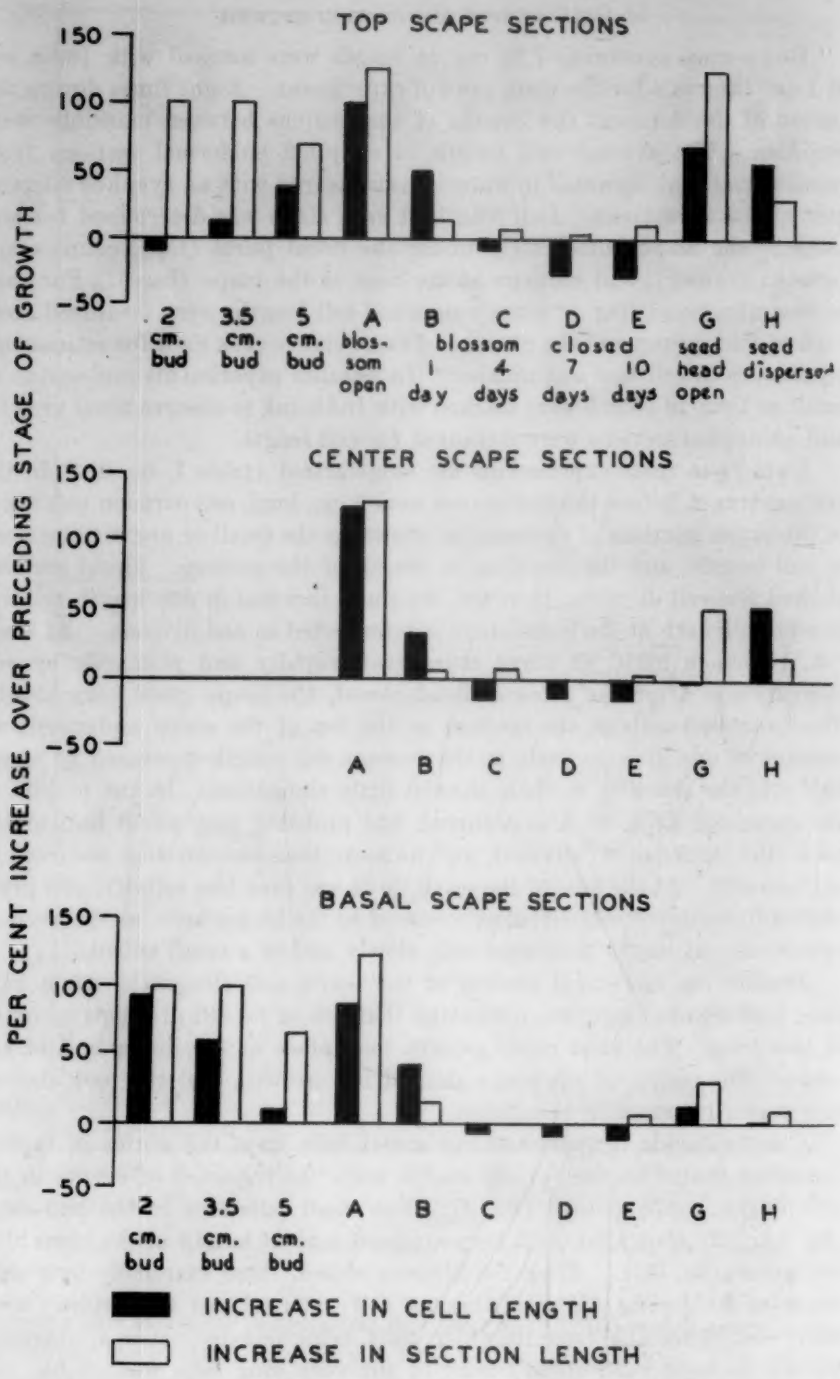

FIG. 2. Elongation of epidermal cells and of marked sections of dandelion scape.

TABLE I

TOTAL ELONGATION AND CELL ELONGATION IN THE DANDELION SCAPE

GROWTH STAGE	TOP				CENTER				BASE			
	SECTION		CELL		SECTION		CELL		SECTION		CELL	
	LENGTH	INCREASE*	LENGTH	INCREASE*	LENGTH	INCREASE*	LENGTH	INCREASE*	LENGTH	INCREASE*	LENGTH	INCREASE*
	cm.	%	μ	%	*cm.*	%	μ	%	*cm.*	%	μ	%
Buds:												
1 cm.	0.1	.....	15.7	.....	0.1	.....	.....	.....	0.1	.....	15.7	.....
2 cm.	0.2	100	14.0	−11	0.2	100	.....	.....	0.2	100	30.1	92
3.5 cm.	0.4	100	16.7	19	0.4	100	.....	.....	0.4	100	46.8	55
5 cm.	0.6	50	23.4	40	0.6	50	53.5	.....	0.6	50	50.1	7
Blossom:												
A, just open	1.4	133	46.8	100	1.4	133	123.6	131	1.4	133	90.2	80
B, just closed	1.65	18	70.2	50	1.5	7	163.7	32	1.65	18	130.3	44
C, closed 4 days	1.8	9	66.8	−5	1.6	7	143.7	−12	1.65	0	123.6	−5
D, closed 7 days	1.9	6	50.1	−25	1.6	0	123.6	−14	1.65	0	116.9	−5
E, closed 10 days	2.2	16	36.7	−27	1.65	3	106.9	−14	1.75	6	103.6	−11
F, closed 13 days	.....	.....	.....	.....	.....	.....	100.2	.....	.....	.....	110.2	.....
Seed:												
G, head open	4.9	123	60.1	64	2.45	48	105.2	−2	2.25	29	116.9	13
H, dispersed	6.3	29	93.2	55	2.9	18	159.7	52	2.4	7	117.3	0

* Increase over previous stage.

the seed head opened (fig. 3F) the cells were again elongating, although they never attained the length they had during the period of blossoming.

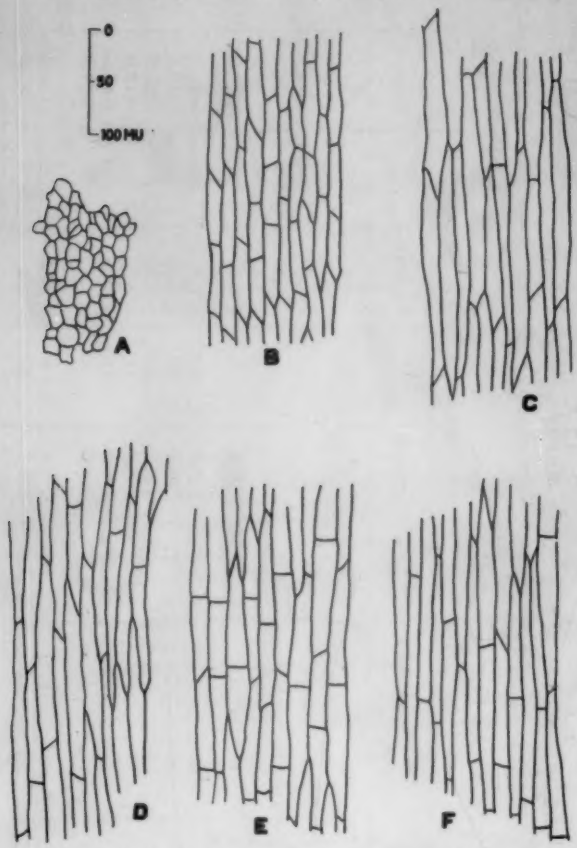

FIG. 3. Epidermal cells from the center of the dandelion scape during growth. Figures were drawn from living material with the aid of the camera lucida. a. Epidermal cells of the 2-cm. bud-scape; b. epidermal cells of the 5-cm. bud-scape; c. epidermal cells of the blossom-scape; d. epidermal cells of the scape several days after blossoming; e. epidermal cells of the scape several days before opening of the seed head; f. epidermal cells of the scape of the open seed head.

CELL ELONGATION IN EXCISED SECTIONS

Sections of scape 1 cm. in length grew very satisfactorily in indoleacetic acid–sucrose solutions in Petri dishes, elongating as much as two or three times in a period of several days under favorable conditions. It was unnecessary to use any sterile precautions, since changing the solutions once a day prevented excessive bacterial contamination for as long as four days. A 1% concentration of sucrose was found satisfactory, higher concentrations shortening the number of days during which it was possible to grow unsterile sections of scape without tissue deterioration. Indoleacetic acid gave

best results at 1 p.p.m. to 5 p.p.m. The addition of sources of nitrogen and phosphorus increased contamination and did not accelerate growth of scape material.

Top sections of bud-scapes showed the greatest increase in length in solution, blossom-scapes elongating moderately but not to the same extent, and the older scapes showing even less growth. The relatively great elongation of the bud-scapes was to be expected, since the cells of such scapes were small, and rapid growth of the intact organ normally occurs at this time. The elongation rate of sections of bud-scape in sucrose-auxin solution was comparable to that which occurred on the plant. Elongation of top sections of the scape of the closed flower, "F," did not approach that attained in the plant, sections elongating only 50% under the most favorable conditions of temperature and indoleacetic acid concentration.

TABLE II

ELONGATION OF SECTIONS AND OF EPIDERMAL CELLS OF THE DANDELION BUD-SCAPE DURING 24 HOURS IN A 1% SUCROSE SOLUTION CONTAINING 5 MG. INDOLEACETIC ACID PER LITER. (AVERAGE OF 10 SECTIONS OR OF 80 CELLS)

TEMPERATURE	FINAL LENGTH* OF SECTIONS	FINAL LENGTH† OF EPIDERMAL CELLS	INCREASE IN LENGTH OF EPIDERMAL CELLS
°C.	cm.	μ	%
0.5	1.10	34.7	37.7
5.0	1.13	33.4	32.5
10.0	1.27	28.7	13.9
14.0	1.50	41.7	65.5
20.5	1.60	42.8	69.8
23.0	1.53	45.7	81.3
27.0	1.87	48.5	92.5
31.0	1.60	50.1	98.8
35.0	1.53	34.7	37.7

* Initial length of sections, 1 cm.
† Initial length of epidermal cells, 25.2 cm.

The elongation of sections and epidermal cells of the dandelion bud-scape during 24 hours in solution at various temperatures is shown in table II. These results are typical of three similar experiments. Although cell division occurred in scapes which remained in solution several days, no evidence of cell division was obtained in the 24-hour period, cell elongation being of the same magnitude as section elongation within the limits of variability. Rise of temperature markedly increased the rate of cell elongation. As increase of temperature does not accelerate physical processes to any marked extent, the large increase of cell elongation rates with rising temperatures indicates that chemical reactions are involved.

Nitrogen and dry weight changes

Nitrogen determinations were made by the semi-micro Kjeldahl method, using essentially the procedure proposed by MA and ZUAZAGA (7). Samples for fresh weight, dry weight, and nitrogen determinations were collected on

the same days that cell lengths were studied, and comparable scape material was used. A comparison of total nitrogen with protein and non-protein nitrogen was made on sections from control parts of scapes at all critical stages, using the method outlined by BLANK and FREY-WYSSLING (3) for separation of protein and non-protein nitrogen.

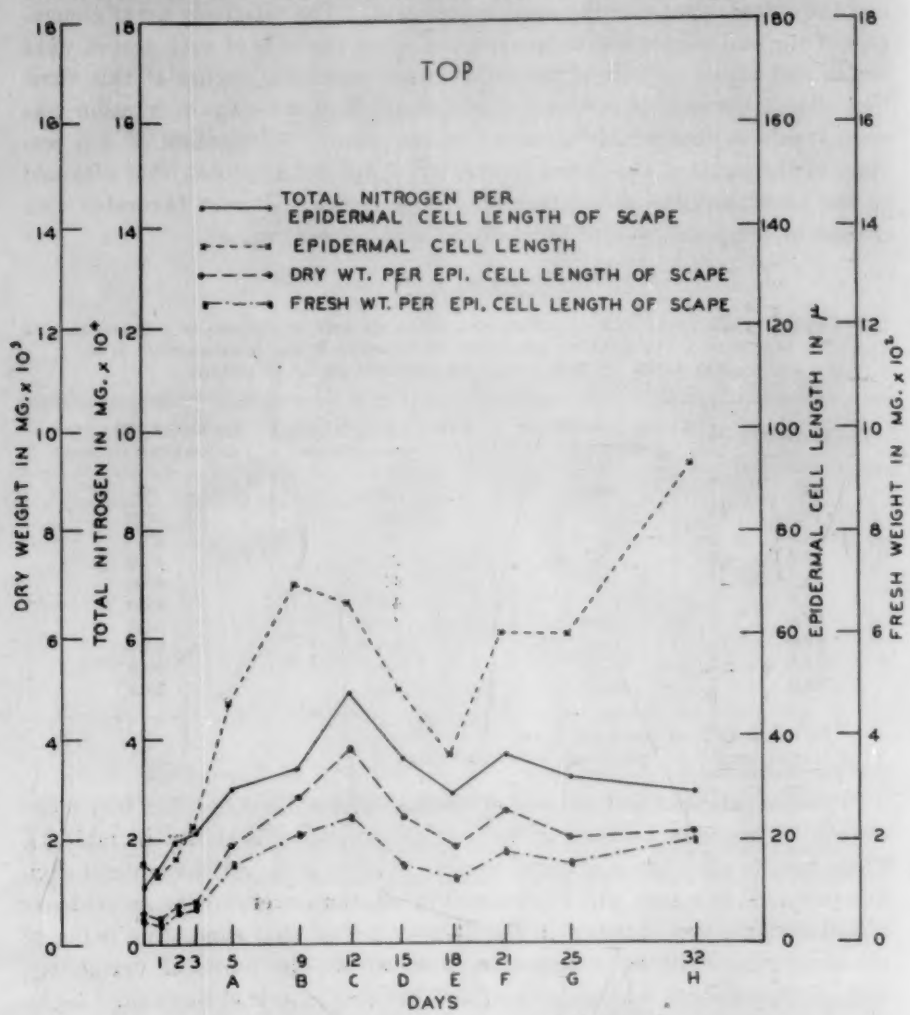
TOP
TOTAL NITROGEN PER EPIDERMAL CELL LENGTH OF SCAPE
EPIDERMAL CELL LENGTH
DRY WT. PER EPI. CELL LENGTH OF SCAPE
FRESH WT. PER EPI. CELL LENGTH OF SCAPE
DRY WEIGHT IN MG. x 10³
TOTAL NITROGEN IN MG. x 10⁴
EPIDERMAL CELL LENGTH IN μ
FRESH WEIGHT IN MG. x 10²
DAYS
1 2 3 5 9 12 15 18 21 25 32
A B C D E F G H

FIG. 4. Total nitrogen, fresh weight, and dry weight, in relation to epidermal cell length at the top of the growing dandelion scape.

The total nitrogen, green weight, and dry weight, changes of three representative regions of the dandelion scape during growth are plotted (figs. 4, 5, 6). In all parts of the scape, total nitrogen in percentage of dry matter was highest in the bud stage, indicating that carbohydrate accumulation was subsequently more rapid than nitrogen accumulation. The amount of pro-

tein nitrogen in the bud-scapes was about 2.5 times as great as the amount of non-protein nitrogen. In the bud the floral parts were developing and the egg cells and pollen were being produced. Nitrogen was undoubtedly moving through the scape into the bud, but nitrogen per epidermal scape cell increased continuously and progressively from the time the scape was

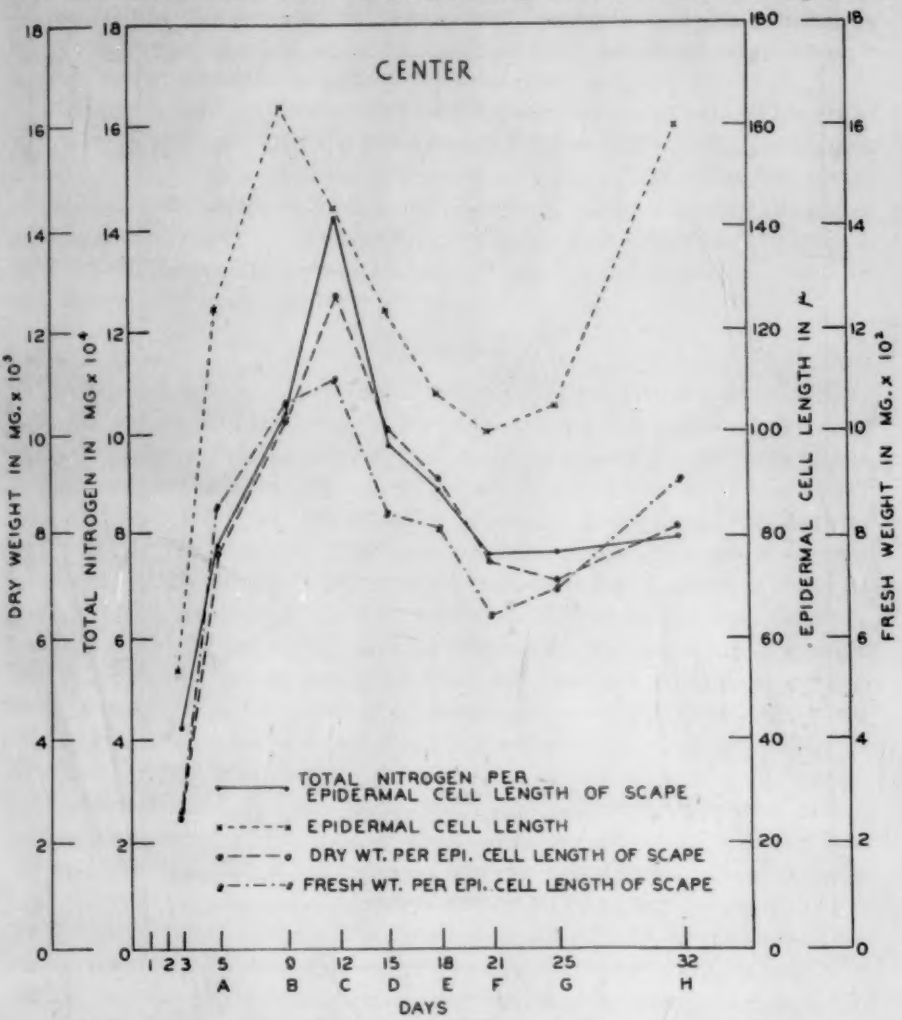
CENTER
DRY WEIGHT IN MG. x 10³
TOTAL NITROGEN IN MG x 10⁴
EPIDERMAL CELL LENGTH IN μ
FRESH WEIGHT IN MG. x 10²
TOTAL NITROGEN PER EPIDERMAL CELL LENGTH OF SCAPE
EPIDERMAL CELL LENGTH
DRY WT. PER EPI. CELL LENGTH OF SCAPE
FRESH WT. PER EPI. CELL LENGTH OF SCAPE
1 2 3 5 9 12 15 18 21 25 32
A B C D E F G H
DAYS

FIG. 5. Total nitrogen, fresh weight, and dry weight, in relation to epidermal cell length at the center of the growing dandelion scape.

first evident until the period of blossoming was well past. The increase of nitrogen per cell was comparable in extent to the rapid increase of epidermal cell length which occurred just before blossoming in all parts of the scape. The ratio of protein to non-protein nitrogen remained constant throughout these stages of growth. Apparently synthesis of protein forms of nitrogen

as well as an accumulation of non-protein forms were occurring during this period.

As the cells began dividing in the scape after blossoming (after embryo development had started), the percentage of nitrogen in the dry matter remained nearly constant. The total amount of nitrogen in the entire scape apparently did not decrease. The amount of nitrogen per cell, however,

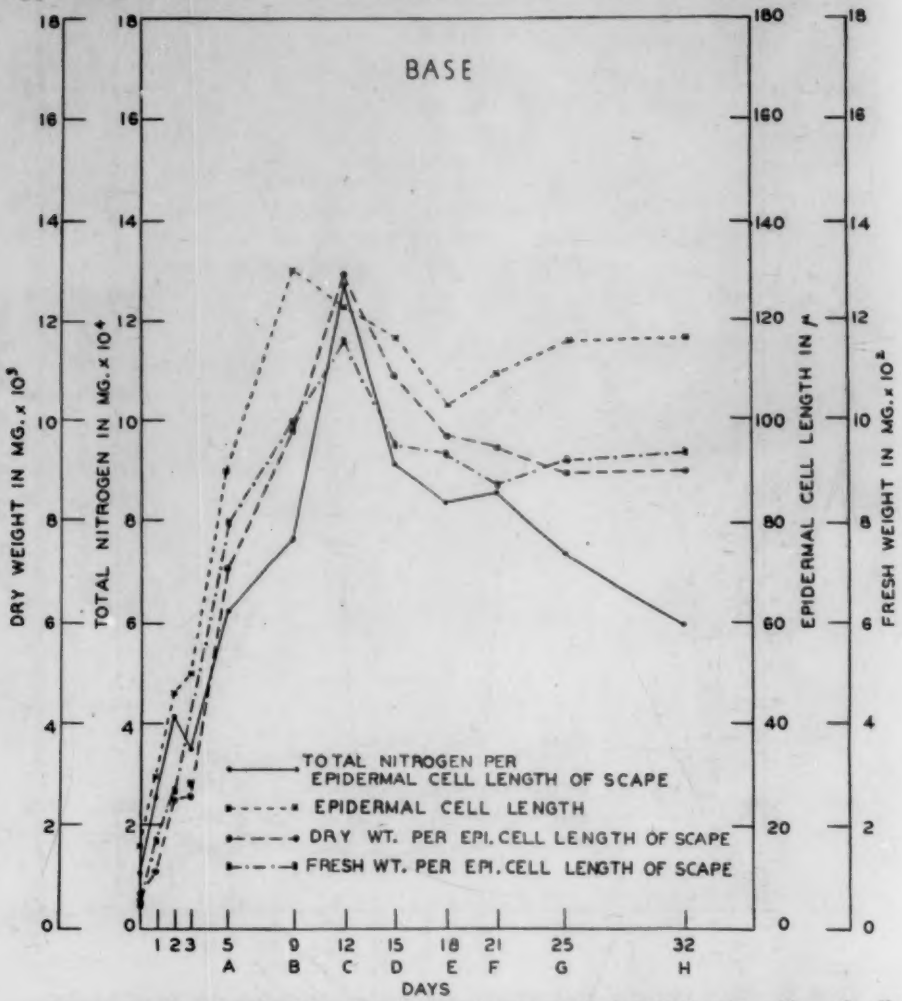
BASE
DRY WEIGHT IN MG. x 10³
TOTAL NITROGEN IN MG. x 10⁴
EPIDERMAL CELL LENGTH IN μ
FRESH WEIGHT IN MG. x 10²
TOTAL NITROGEN PER EPIDERMAL CELL LENGTH OF SCAPE
EPIDERMAL CELL LENGTH
DRY WT. PER EPI. CELL LENGTH OF SCAPE
FRESH WT. PER EPI. CELL LENGTH OF SCAPE
1 2 3 5 9 12 15 18 21 25 32
A B C D E F G H
DAYS

FIG. 6. Total nitrogen, fresh weight, and dry weight, in relation to epidermal cell length at the bottom of the growing dandelion scape.

decreased with division, and as the ratio of protein to non-protein nitrogen still remained the same as in the bud or blossom scape, it appears that synthesis of protein nitrogen during division of the scape cells probably did not occur to any extent at this time.

During the period of rapid cell elongation which preceded and accom-

panied the opening of the seed head (roughly stages "E" to "G") the nitrogen per epidermal cell showed no increase. There was, in fact, some indication of a decrease of nitrogen per cell at this time, especially at the bottom of the scape (fig. 6) which suggests a translocation of nitrogen out of the scape cells. The ratio of protein to non-protein nitrogen which had been constant at all preceding stages, now decreased, indicating that proteins were being digested, and probably that the products of digestion were moving out of the scape into the developing fruit.

The dry weight and fresh weight changes during the growth of the scape are plotted together with the nitrogen and epidermal cell length changes (figs. 4, 5, 6). Dry weight per cell increased greatly during the first period of rapid cell elongation and remained practically constant during the period of slow scape growth after blossoming. During both periods of cell elongation the increase of fresh weight per cell was somewhat greater than the increase of dry weight per cell.

Discussion

In general, epidermal cell length and protein nitrogen, non-protein nitrogen, dry weight, and fresh weight per cell increased and decreased at similar rates until the epidermal cells began to elongate rapidly for the second time, unaccompanied by a corresponding increase of total nitrogen or dry weight, and with only the fresh weight and non-protein nitrogen per cell showing any tendency to increase. The evidence that protein nitrogen formation occurred rapidly during cell elongation at flowering and only to a small extent, if at all, during divisions of cells after flowering was somewhat surprising in view of the widely existing impression that cell division involves great increase of protoplasmic material and that cell elongation involves little or none. The formation of proteinaceous material during cell elongation has been observed to occur in the coleoptile of *Zea mays* (**5**) and in the hypanthium of *Oenothera acaulis* (**3**). In studies of growth of broccoli cotyledons evidence has also been found for protoplasm formation during cell enlargement. Cotyledons of seedlings germinated in sand and later transplanted into fertile soil enlarged three to four times after transplantation, whereas the cotyledons of seedlings remaining in sand showed little or no growth. No cell division was involved in this growth, cotyledon and cell enlargement being of the same order. In a typical experiment the cotyledon volume increased 3.71 times, total nitrogen 2.30 times, protein nitrogen 1.74 times, and non-protein nitrogen 2.73 times following transplantation. These data indicate that some substance, possibly available nitrogen, supplied by the more fertile medium was capable of reviving and extending cell enlargement in the absence of cell division in the cotyledons. The rapid rate of protein accumulation in these young, enlarging cells of the broccoli cotyledon is interesting, but a direct relationship between protein synthesis and cell elongation in general is not established, since cell elongation in intact dandelion scapes at the time of seed maturity occurred without increase of

protein nitrogen per cell, and sections of young scapes in sugar and hormone solutions grew rapidly by cell elongation without additional nitrogen. The bud-scapes which elongated in solutions in the absence of an external source of nitrogen, however, had cells which were small and rich in protoplasm as elongation began. If cell enlargement occurs through the stimulation of a basic protoplasmic process which in turn brings about secondary effects (**4**, **11**), it may be that synthesis of protoplasm is not the direct means of bringing about cell enlargement, but that a sufficient supply of protoplasm is prerequisite to the initiation of the process. A relationship between protein synthesis and auxin synthesis may also be involved (**1**).

Since elongation may accompany cell division in cells of the dandelion scape, or the two processes may occur independently, it would be of interest to discover which conditions stimulate cells to divide, which stimulate cells to elongate and under what conditions both processes occur more or less simultaneously. The two processes are undoubtedly more closely related and more nearly subject to the same stimulating mechanism than has been generally suspected. GOODWIN and STEPKA (**6**) have shown that the region of most active cell divisions in Phleum roots is also the region of most active cell elongation. Experiments reported in this paper have indicated that chemical reactions and synthesis of protoplasm are found in enlarging as well as dividing cells, and, in fact, that cell division in the dandelion scape does not necessarily involve protein synthesis. It may be that the difference between the mechanism stimulating division and that stimulating enlargement of cells is very slight.

The prominence of protein synthesis in the first period of rapid cell elongation in the dandelion scape and its absence in the second suggest that the stimulus for cell enlargement may be either internal or external to the enlarging cell. If this stimulus be hormonal, it may be postulated either that protein and hormone synthesis within the cell (**1**, **2**) stimulates enlargement of the same cell, or that an external supply of hormone from active buds, leaves or fruits above may accomplish the same result. In the broccoli experiments, growth of the entire plant was checked by nutrient deficiencies and cell enlargement in the cotyledons stopped, to be resumed when a supply of soil nutrients made possible the general resumption of protein and presumably of hormone synthesis.

The effect of increasing temperature in accelerating the rate of cell elongation in sections of the scape clearly indicates the presence of limiting chemical reactions either in the wall or in the protoplasm and furnishes additional evidence of the complexity of cell enlargement processes.

Summary

A comparison of organ growth and cell growth was made in dandelion scapes of field plants. Cells were simultaneously dividing and increasing in length until the blossom began to open. At this time scape growth was accelerated and few or no cell divisions occurred. After the blossom closed,

scape growth was very slow and cells were reduced in length through cell division. As the seed head opened, the scape again grew rapidly by cell elongation. During the first rapid elongation the whole scape grew uniformly, although there were probably differences in the relative roles of cell division and elongation in the upper and lower portions, but during the second rapid elongation most of the growth took place at the upper end of the scape.

One-centimeter sections of small bud-scapes elongated rapidly in nonsterile, indoleacetic acid-sucrose solutions, tripling their length in several days under the most favorable conditions. The rate of cell elongation was accelerated by increasing temperatures from 0° to 25° C. The importance of chemical reactions in cell enlargement is indicated.

Total nitrogen as well as protein nitrogen, fresh weight, and dry weight per cell increased during the first rapid growth of the scape. During the second period of rapid elongation, dry weight and protein nitrogen per cell decreased, and fresh weight and non-protein nitrogen per cell increased only slightly. Protein synthesis may or may not accompany cell elongation in scape tissue, and the hypothesis is advanced that cell enlargement may be stimulated either by hormones produced during protein synthesis within the enlarging cell, or by hormones moved from a center of cell activity external to the stimulated cells.

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SOME EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON FLOWER FORMATION AND RUNNER PRODUCTION IN THE STRAWBERRY

H. T. HARTMANN

(WITH THREE FIGURES)

Received April 12, 1947

The regional and geographical adaptation of strawberry varieties is known to be influenced by day-length and temperature. As DARROW and WALDO (**3**) have shown, the ordinary varieties of strawberries are short-day plants, initiating flower buds when the light period per day approximates 10 hours or less. According to DARROW (**2**), temperature exerts a modifying influence on the response of the strawberry to the photoperiod.

In this investigation the response of the strawberry to certain temperature and day-length treatments was studied under controlled environmental conditions. The strawberry is well adapted for use in studies of this kind as it is known to have definite photoperiodic responses which are influenced by temperature. These responses are judged by criteria that are definite and easily measured; namely, the formation of flower clusters when the plant is in the reproductive state and the production of runners when it is in the vegetative condition. In addition the strawberry can be propagated vegetatively by runners, thereby furnishing plant material of the same genetic constitution for experimental treatment.

The equipment used in the present investigation consisted of a set of four environment-control cabinets, described in detail by HARTMANN and MCKINNON (**5**). With these cabinets the temperature could be maintained at desired levels by electric heaters and refrigeration units, both thermostatically controlled. The plants were grown under banks of fluorescent lamps, which maintained a light intensity of about 500 foot-candles at the level of the plants. The day-length conditions could be adjusted at will and were automatically maintained by a time switch.

Methods and results

To test the response of the Missionary strawberry to short and long light periods under the controlled environmental conditions used in this investigation a preliminary experiment was carried out. Twenty plants of this variety, which had been growing in long days and were definitely in a vegetative state, were placed in the cabinets on July 9, 1942. Ten plants were maintained under "short-days" (10 hours) and ten were put under "long days" (15 hours). They were grown in sandy loam in gallon cans. Flower and runner counts were made at intervals from July 23 to October 15. The results are given in table I and definitely show this strawberry variety to be

vegetative under a 15-hour photoperiod and reproductive with a 10-hour photoperiod.

Experiment I. The effect of constant temperature in comparison with fluctuating temperature on the photoperiodic response of the strawberry

It was desired to conduct experiments on photoperiodic response with plants maintained under constant temperature conditions. Under natural conditions plants are, of course, subjected to a daily fluctuation of temperature in addition to the daily alternation of light and darkness. That is, the temperature is higher during the light period than during the dark period.

TABLE I

The effect of 10- and 15-hour photoperiods at a constant temperature of 70° F. on vegetative and reproductive growth of the Missionary strawberry. (Plants placed under test conditions July 9, 1942)

Date	Number of runners removed from 10 plants		Number of flowers produced by 10 plants	
	10-hour days	15-hour days	10-hour days	15-hour days
July 23	4	18	0	0
August 6	0	10	0	0
August 20	0	24	0	0
September 3	0	24	0	0
September 17	0	17	0	0
October 1	0	23	13	0
October 6	0	.....	24	0
October 9	0	.....	32	0
October 13	0	.....	29	0
October 15	0	20	41	0
Total	4	136	.....	.....

This experiment was designed to determine whether this daily temperature fluctuation is a factor influencing the production of flowers in the strawberry. Two conditions were maintained: (1) short days (10 hours) with the temperature constant at 70° F.; (2) short days (10 hours) with a temperature of 80° F. during the light period and 60° F. during the dark period. Thirty-six plants of the Missionary variety, which had been maintained under long days and were in a vegetative state, were placed under each of these conditions January 11, 1946. Flower counts were started March 19. In recording the results (flower formation), the plants were considered to be in the reproductive state when there was the first macroscopic evidence of flower-bud formation. This condition is determined by the appearance of a cluster of small flower buds in the crown of the plant. Later this flower cluster emerges on the end of a flower stalk or peduncle. Fruit set was recorded when there was the first indication that the fleshy receptacle was swelling. The fruit was considered ripe when it attained a solid red color. The results of this test are given in table II.

All plants under both conditions formed flowers and set fruits that ripened. This occurred earlier under fluctuating than under constant temperature. The difference in time of flower differentiation in the two groups is small. Nearly half the plants under the fluctuating temperature had, however, set fruits before any in the other group had done so; some ripe fruits occurred three weeks earlier at fluctuating than at constant temperature.

EXPERIMENT II. THE PHOTOPERIODIC RESPONSE OF THE STRAWBERRY UNDER ARTIFICIAL CONDITIONS OF (1) LONG DAYS WITH LONG NIGHTS AND (2) SHORT DAYS WITH SHORT NIGHTS

It was shown in the preliminary test and in Experiment I that the strawberry becomes reproductive with a light period of 10 hours and a dark period

TABLE II

COMPARISON OF FLOWER FORMATION, FRUIT-SETTING, AND FRUIT-RIPENING OF MISSIONARY STRAWBERRY PLANTS GROWN UNDER CONDITIONS OF CONSTANT TEMPERATURE AND FLUCTUATING TEMPERATURE

DATE	NUMBER OF PLANTS FORMING FLOWERS (36 TOTAL)		NUMBER OF PLANTS SETTING FRUITS (36 TOTAL)		NUMBER OF RIPE FRUITS PICKED	
	60° TO 80° F.	70° F.	60° TO 80° F.	70° F.	60° TO 80° F.	70° F.
March 19	10	7	0	0	0	0
March 27	25	21	6	0	0	0
April 1	28	21	6	0	0	0
April 9	31	30	14	0	0	0
April 12	33	30	19	8	0	0
April 18	35	32	25	22	7	0
April 24	36	34	30	22	3	0
April 28	36	35	31	25	17	0
May 3	36	35	34	32	16	13
May 6	36	36	35	34	23	26
May 10	36	36	36	36	23	30

of 14 hours per day at a constant temperature of 70° F. It is possible that the initiation of the reproductive condition may be due to the short (10-hour) light period, the long (14-hour) dark period, or the combination of the two.

This experiment was arranged to study the effect of the short light period and the long dark period on flower formation in the strawberry. Two of the four cabinets had the light-controlling time clock set to provide a 20-hour cycle: 10 hours of light followed by 10 hours of darkness, etc. This gave the short light period, but not the long dark period. The time clock controlling the lights in the remaining two cabinets were arranged to provide a 28-hour cycle; that is, 14 hours of light followed by 14 hours of darkness, etc. This resulted in the long dark period, but not the short light period.

Forty-two plants were put in the cabinets under each set of conditions

on May 13, 1946, having been maintained previously under long-day conditions. The temperature was held constant at 70° F. Vegetative response was measured by counts of runners produced, and reproductive response by flower clusters formed. The results of these measurements appear in table III.

The plants in both series continued to produce runners from the time they were placed under the test conditions on May 13 until about June 14, a period of 4½ weeks. This situation was due to runner initials which were formed while the plants were under long days previous to being placed in the conditions of the test. From June 14 to the conclusion of the test there

TABLE III

THE EFFECT OF 20-HOUR AND 28-HOUR PHOTOPERIODIC CYCLES ON RUNNER PRODUCTION AND FLOWER FORMATION IN THE MISSIONARY STRAWBERRY. (PLANTS PLACED UNDER TEST CONDITIONS MAY 13, 1946)

DATE	VEGETATIVE RESPONSE (RUNNERS REMOVED FROM 42 PLANTS)		REPRODUCTIVE RESPONSE (NUMBER OF PLANTS WITH FLOWER CLUSTERS; 42 PLANTS TOTAL)	
	28-HOUR CYCLE	20-HOUR CYCLE	28-HOUR CYCLE	20-HOUR CYCLE
May 23	25	32	0	0
May 29	36	26	0	0
June 3	27	12	0	0
June 6	11	17	0	1
June 12	6	1	1	2
June 14	0	2	1	2
June 22	2	2	2	2
July 1	2	0	2	2
July 13	3	0	6	2
July 16	8	0	6	4
July 23	19	0	17	21
July 29	24	0	22	26
August 7	44	0	23	41

was a striking difference in the production of runners. The plants maintained under conditions of short light periods and short dark periods (20-hour cycle) ceased runner production altogether, whereas the plants under long light and long dark periods (28-hour cycle) returned to runner formation. The plants under the last-named condition had a temporary decline in formation of runner initials, probably occurring when they were changed from the normal 24-hour cycle to the artificial 28-hour cycle.

As for reproductive response, the plants in the two groups behaved similarly in their production of flowers, except for the reading made on August 7. At this time, in the 20-hour group, 41 out of 42 plants had flowers, whereas in the 28-hour group only a little over half the plants had flowers appearing. Plants grown with long light and long dark periods (28-hour cycle) produced runners and flowers simultaneously. Whenever flower clusters were formed under any of the conditions, they continued to develop, and set fruit.

EXPERIMENT III. PHOTOPERIODIC INDUCTION IN THE STRAWBERRY

In the occurrence of photoperiodic induction, plants can be placed under those conditions of daily light duration which are favorable for initiation of floral primordia and, if kept there sufficiently long, will continue flower development even though they are subsequently moved to photoperiodic conditions unfavorable for flower formation.

In this experiment strawberry plants of the Missionary variety were obtained which originated as runner plants from a field planting that had been under long-day (midsummer) light conditions. They were potted and

TABLE IV

VEGETATIVE AND REPRODUCTIVE RESPONSE OF PLANTS HELD FOR DIFFERENT INTERVALS OF SHORT-DAY (10 HOURS) TREATMENTS FOLLOWED BY SUBSEQUENT EXPOSURE TO LONG-DAYS (15 HOURS)

DATE	NUMBER OF DAYS EXPOSED TO SHORT PHOTOPERIODS			
	3 DAYS*	7 DAYS†	21 DAYS‡	38 DAYS§
VEGETATIVE RESPONSE (RUNNERS REMOVED FROM 10 PLANTS)				
August 20	7	3	6	4
August 27	11	9	5	2
September 6	13	10	3	2
September 16	11	10	0	0
September 23	10	11	6	0
September 30	11	6	4	0
October 7	6	3	0	0
October 14	7	5	2	0
October 21	9	8	2	8
October 26	7	5	4	2
REPRODUCTIVE RESPONSE (NUMBER OF PLANTS WITH FLOWER CLUSTERS; 10 PLANTS TOTAL)				
September 30	0	4	9	6
October 7	0	5	9	10
October 14	0	5	10	10
October 21	0	5	10	10
October 26	0	5	10	10

* Moved to long-day conditions August 13.
† Moved to long-day conditions August 17.
‡ Moved to long-day conditions August 31.
§ Moved to long-day conditions September 17.

placed in the cabinets on August 10, 1946, under short-day conditions (10 hours light and 14 hours darkness per 24 hours). The forty plants used were divided into four groups of ten plants each. Each group remained in the short-day cabinets for a different period of time, then was removed to long days (15 hours of light and 9 hours of darkness per 24 hours). The short-day time periods for the four groups were as follows: (1), 3 days; (2), 7 days; (3), 21 days; (4), 38 days. Results of this experiment were obtained by making counts of runner production and flower formation at intervals. These counts are given in table IV. Throughout this experiment the plants were held at a constant temperature of 70° F.

Floral primordia were initiated when the plants were exposed to 7 photoperiodic cycles consisting of 10 hours of light and 14 hours of darkness per 24 hours. These primordia continued to develop even though the plants were subsequently placed under long-day conditions (15 hours light and 9 hours darkness per 24 hours). Exposure to 3 short-day cycles failed to result in initiation of floral primordia. This fact would indicate that the critical number of short-day cycles required for initiating floral primordia in the Missionary strawberry lies between 3 and 8. Exposures to longer "induction periods" (21 days and 38 days) also resulted in initiation and development of flower clusters. An inverse relation was apparent between runner production and flower formation. Those plants initiating and producing the greatest numbers of flower clusters developed the fewest runners.

Experiment IV. Translocation of a flower-forming stimulus in the strawberry

Theories have been proposed (**1**, **6**, **7**, **8**) that the floral primordia in plants may be initiated by a flower-forming stimulus which is manufactured in the leaves when they are under the proper environmental conditions. This stimulus is presumably translocated to the growing points of the plant, where it results in the differentiation of floral parts. With the equipment available for this investigation, this theory in regard to the strawberry could be tested.

Two types of experiments were performed: one with the leaves, the other with runners. In the test with leaves the plant itself (crown and roots) was maintained under long-day conditions, but the leaves (one or more) were placed in short days. This arrangement was made by cutting an opening in the partition between the short-day and long-day cabinets and letting the leaf petioles extend through the opening. The openings were light-proofed by packing around the petioles with felt. The Missionary variety was used, and the temperature was held constant at 70° F.

Hamner and Bonner (**4**) have shown that fully expanded leaves of the short-day plant Xanthium, exposed to long photoperiods, inhibited the transmission of the stimulus through the stem to which the leaves were attached. Stout (**9**), working with the sugar beet, a long-day plant, believed that the substance conducive to reproductive development might be translocated with the carbohydrates.

In view of such evidence this test was subdivided into three groups: (1), all the leaves of each plant were maintained in short days; only the roots, crown, and part of the petioles were contained in the long-day cabinets; (2), fifty % of the leaves were held under short-day conditions, while the remaining fifty % were in the long-day cabinets together with the roots and crown of the plant; (3), one leaf only was placed in the short-day cabinet, the remainder of the plant being under long days. Five plants were used in each group and on August 10, 1946, were arranged in the manner described above.

Results were obtained at intervals throughout the test by counts of runner production and flower formation. These results are given in table V.

Plants held in long photoperiods became reproductive when portions of the leaf area were subjected to short days. The number of flower clusters produced was in direct ratio to the percentage of the total leaf area placed under short-day conditions. The formation of runners was in inverse ratio to the percentage of total leaf area held under short-day conditions. Plants with the entire leaf area exposed only to long days formed no flower clusters, but produced more runners than did any other group among the four.

TABLE V

THE EFFECT OF SHORT PHOTOPERIODS ON PORTIONS OF THE LEAF AREA OF PLANTS MAINTAINED UNDER LONG-DAY CONDITIONS IN REGARD TO THEIR VEGETATIVE AND REPRODUCTIVE RESPONSE. (PLANTS PLACED UNDER INDICATED CONDITIONS AUGUST 10)

DATE	PORTION OF THE LEAF AREA HELD UNDER SHORT PHOTOPERIODS			
	0 LEAVES (CONTROL)	1 LEAF	50% OF LEAF AREA	100% OF LEAF AREA
VEGETATIVE RESPONSE (RUNNERS REMOVED FROM 5 PLANTS)				
August 20	3	3	2	4
August 27	7	5	2	0
September 6	6	5	3	1
September 16	4	3	0	0
September 23	6	4	0	0
September 30	5	6	1	0
October 7	7	4	1	0
October 14	3	3	1	0
October 21	6	3	0	0
October 26	4	3	0	0
Total	51	39	10	5
REPRODUCTIVE RESPONSE (NUMBER OF PLANTS WITH FLOWER CLUSTERS; 5 PLANTS TOTAL)				
September 23	0	0	0	0
October 7	0	0	0	0
October 14	0	0	1	1
October 21	0	1	2	4
October 26	0	1	2	4

In the second type of test for a flower-forming stimulus in the strawberry, runner plants were used which were attached to the parent plant. The parent plants were secured from a field planting growing under long-day (mid-summer) conditions; each plant selected had a long runner attached. The parent plants were transplanted to gallon cans and placed in the short-day cabinets. The runners were threaded through an opening in the partition into the long-day cabinets. There they were "layered" in soil in pots, where they rooted and grew into plants. Thus, after the beginning of the test on August 10, runner plants were continuously grown under long days, while the parent plants were under short days. Figure 1 shows the arrangement of the parent plant and runner plant in relation to the partition between adjacent cabinets. Half of the runner plants were kept de-

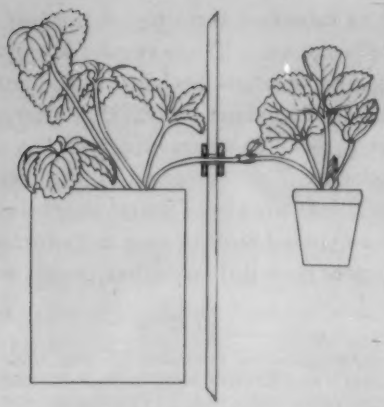

FIG. 1. Method of arranging parent plant and attached runner plant so that each could be maintained under different photoperiodic conditions.

foliated, whereas in the other half the leaves developed normally. By this means a more rapid movement of materials to the defoliated plants from the parent plants seemed likely to occur. The Missionary variety was used in this test, and the temperature was kept constant at 70° F. Counts of runner production and flower formation, taken at intervals, are given in table VI.

TABLE VI

VEGETATIVE AND REPRODUCTIVE RESPONSE OF RUNNER PLANTS GROWING UNDER LONG PHOTOPERIODS (15 HOURS) WHEN ATTACHED BY RUNNERS TO PARENT PLANTS MAINTAINED UNDER SHORT PHOTOPERIODS (10 HOURS).
(TREATMENT STARTED AUGUST 10, 1946)

DATE	RUNNER PLANTS IN LONG DAYS ATTACHED TO PARENT PLANTS IN SHORT DAYS		CONTROL PLANTS GROWING IN	
	DEFOLIATED	NON-DEFOLIATED	LONG DAYS	SHORT DAYS
VEGETATIVE RESPONSE (RUNNERS REMOVED FROM 5 PLANTS)				
August 20	0	0	3	2
August 27	0	0	7	0
September 6	1	0	6	0
September 16	0	1	4	0
September 23	0	7	6	0
September 30	0	2	5	0
October 7	0	1	7	0
October 14	0	2	3	0
October 21	0	0	6	0
October 26	0	0	4	0
Total	1	13	51	2
REPRODUCTIVE RESPONSE (NUMBER OF PLANTS WITH FLOWER CLUSTERS; 5 PLANTS TOTAL)				
September 30	0	1	0	0
October 7	0	2	0	0
October 14	1	2	0	5
October 21	1	3	0	5
October 26	3	3	0	5

The runner plants used were necessarily younger than the long and short-day control plants, which were of the same age as the parent plants.

As shown by the data given in table VI and by the appearance of typical plants in figure 2, runner plants growing under long-day conditions formed flower clusters; provided, however, that they remained attached by runners to plants growing under short-day conditions. Flower clusters formed on those runner plants whose leaves were removed as well as on the non-defoliated plants. The latter were more vigorous, however, and initiated flower clusters sooner, or at least developed more rapidly. This situation might be expected, since these plants would have a greater carbohydrate supply than the plants that depended entirely on carbohydrate movement through the runner from the parent plant. Not all of the runner plants formed flower

FIG. 2. Appearance of typical plants when grown under short-day and long-day conditions. The runner plant (growing in long days) produced flowers because of its attachment to the parent plant (growing in short days).

clusters even though they were attached by runners to plants growing in short days. It was noted that in such plants the runners connecting the two plants turned brown and shrivelled; these runners were probably incapable of translocating food materials or any floral-inducing stimulus.

The plants attached by runners to parent plants growing in short days produced fewer runners than the control plants grown under long days. Allowing leaves to develop on the attached runner plants (growing in long days) increased their vegetative response, as is shown by the increased production of runners in comparison with those attached runner plants that were defoliated.

Discussion

Although flower buds appeared at about the same time under conditions of constant temperature and fluctuating temperature, there was apparently

a constant and consistent hastening of flower and fruit development on plants grown at the fluctuating temperatures used. The plants in the 60°-to-80° F. group had a lower "average temperature" than the plants held constantly at 70°: they were at 80° for 10 hours, at 60° for 14 hours. Nevertheless, the plants in the 60°-to-80° group had a greater developmental rate than those in the 70° group. This condition may be explained by the temperature coefficient of at least 2, characteristic of most plant-cell reactions, which would cause an accelerated metabolism of the plant cells at the 80° F. temperature. For example, if x equals the rate of cell metabolism per hour at a temperature of 70°, the plants held at this temperature would have a relative metabolic rate of 24x in one day. The plants in the 60°-to-80° temperature group would have a metabolic rate of 2x for the 10 hours at 80°, or 20x; they would also have a metabolic rate of 0.5x for 14 hours at 60°, or 7x. This would then total 27x for the 60°-to-80° F. group in comparison with the 24x rate for the 70° group. Thus the plants held under the fluctuating temperature conditions (60°to 80° F.) could be expected to show more rapid developmental characteristics, because of the greater daily metabolic rate.

The rate of photosynthesis would supposedly be the same for the two groups of plants even though the latter were held under different temperatures (70° and 80° F.) during their light periods. As the light intensity used was relatively low (about 500 foot-candles) the "light reaction" was presumably the limiting factor in the photosynthetic process. As this reaction has a temperature coefficient of 1, the different temperatures involved would therefore not influence the photosynthetic rate.

Under field conditions the Missionary strawberry will form runners abundantly during summer with 14 hours of light daily, accompanied, of course, by 10 hours of darkness. Under the conditions used in the second experiment, plants produced runners with a 14-hour daily light period together with a 14-hour dark period, the 14-hour dark period being substituted for the accompanying short (10-hour) dark period found in nature. When a short dark period (10 hours) was used together with a short light period rather than the normal accompanying long light period, no runners were produced. From these facts one may conclude that the long light period appears to be necessary for runner production (vegetative response) rather than the short dark period.

In regard to the reproductive response of the plants used in Experiment II, it is noted that the plants produced flower clusters under both the 28-hour and the 20-hour cycles. Plants grown with long light and long dark periods (28-hour cycle) produced runners and flowers simultaneously.

Four photoperiodic combinations of day and night are possible: (1), long days and short nights; (2), short days and long nights; (3), long days and long nights; and (4), short days and short nights. The first two occur in nature as a 24-hour cycle. Flower formation occurs in the Missionary strawberry under natural conditions only in the second case (short days and long

nights). As table III indicates, flower formation occurred also under the the two artificial conditions: long days with long nights, and short days with short nights. Thus, of the four possible photoperiodic combinations, in only one—long days and short nights—does flower formation fail to occur. If a hormonal mechanism is responsible for flower formation it is possible that a substance may be produced during the light period which must be utilized in a subsequent reaction during the following dark period for flowering to occur. If the dark period is of insufficient duration, in relation to the length of the light period, for complete utilization of the substance, the result may be an accumulation that inhibits flower formation. In this investigation flower formation occurred when the light and dark periods were of equal

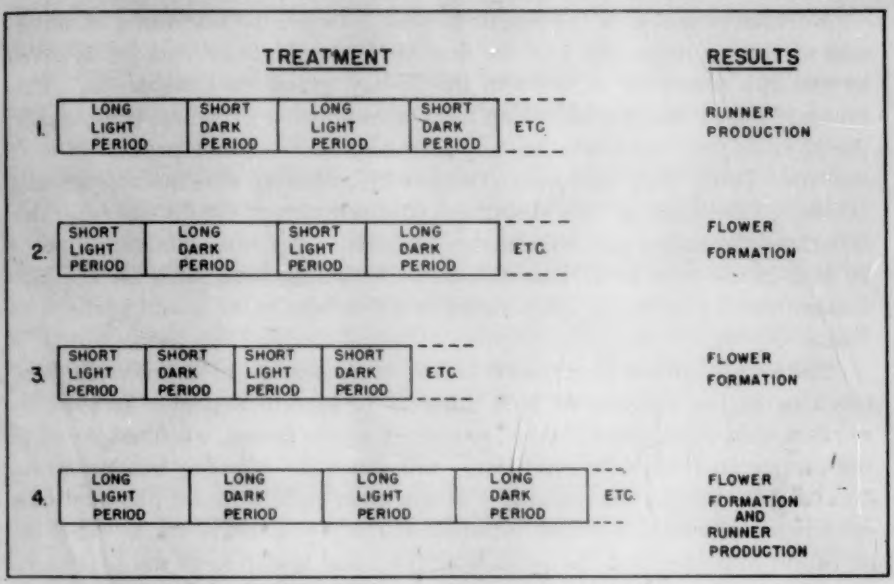

FIG. 3. Vegetative and reproductive response of the Missionary strawberry to the four possible combinations of light and dark periods. Treatments 1 and 2 can occur naturally as a 24-hour cycle. Treatments 3 and 4 are artificial.

length, regardless of whether they were long or short. Under natural conditions flower formation in the strawberry takes place, within limits, when the dark period is longer than the light period, but not when the dark period is shorter than the light period. This situation is shown graphically in figure 3.

In the experiment on photoperiodic induction in the strawberry, the first flower clusters appeared at the same date (September 30) in all the three groups that became reproductive. The plants were all placed under the short-day conditions at the same date (August 10). Thus about 50 days were required, after the initial exposure to photoperiodic conditions conducive to development of the reproductive state, before flower clusters became visible. The first microscopic appearance of the initiation of floral

primordia (although not observed here) would occur, of course, some time before the date of macroscopic visibility.

Runners initiated by the previous exposure to long days failed to appear on plants in the 38-day group after September 6. This date was 27 days after these plants were moved (on August 10) from long days (conducive to runner formation) to short days (inhibiting runner formation). Assuming that initiation of runner primordia ceased immediately after the plants were placed in short days, these observations would indicate that about 27 days are required after the start of physiological conditions leading to initiation of runner primordia, before the primordia become macroscopically visible.

Further evidence on the length of time between the beginning of initiation of runner primordia and the first visible evidence of runners is given by the appearance of runners in the 38-day group on October 21. This group of plants was placed in long days on September 17, when, presumably, the physiological conditions leading to initiation of runner primordia were started. Thirty-four days later (October 21), runners were macroscopically visible. The length of time elapsing from beginning of conditions favorable to runner formation and macroscopic visibility was thus established to be 27 days in one case, 34 days in the other. This time interval is for the Missionary variety only; for other varieties a different value would perhaps be found.

The results of the experiment on the translocation of a flower-forming stimulus in the strawberry lend support to certain evidence secured by workers with other plant species; namely, that the leaves, when subjected to the proper photoperiodic conditions, will cause the plant to become reproductive even though the remainder of the plant is held under photoperiodic conditions unfavorable to the initiation of the reproductive state. This observation indicates that the portion of the plant sensitive to the photoperiodic treatment is the leaf. It would seem justifiable to assume that a stimulus is translocated from the leaves to the growing point of the plant to cause initiation of the reproductive state. To explain why more of the plants became reproductive when a higher percentage of the leaf area was held under short days, one may suggest that a larger quantity of the flower-forming stimulus is perhaps produced by the greater number of leaves under short days.

In experiment IV plants were induced to become reproductive even though they were grown under photoperiodic conditions (long days) definitely known to be unfavorable to the development of the reproductive state in the strawberry. This reproductive condition was caused by the attachment of these plants by runners to other plants which were growing under photoperiodic conditions (short-days) favorable to the initiation of the reproductive state and which were themselves producing flowers. These conditions are illustrated in figure 2. The runner plants used in this experiment were undoubtedly dependent upon the parent plants, at least early

in their existence, for food materials. Those runner plants which were kept defoliated were more dependent for materials upon their parent plants than the runner plants whose leaves developed normally. As table VI shows, however, both groups became reproductive, developing flower clusters, although the non-defoliated plants formed flower clusters more rapidly. STOUT, in his work (9) with the sugar beet, concluded that the reproductive-inducing substance may be translocated with the carbohydrates. According to the results of the present investigation, the substance inducing reproduction moved equally well under conditions where different amounts of carbohydrate movement could be expected. The flower-inducing stimulus may have been translocated, however, with the carbohydrate stream very early in the life of the runner plants, when both groups were largely dependent upon their parent plants for food materials.

Generally the production of runners (vegetative state) and the formation of flower clusters (reproductive state) in the strawberry seem to be antagonistic. That is, conditions favoring one tend to inhibit the other. This situation occurred in all cases in this investigation with the exception of the one test in which the plants were grown under the artificial conditions of long days (14 hours) and long nights (14 hours). Here the same plants produced runners and flower clusters simultaneously. This observation would indicate, therefore, that the occurrence of the vegetative state does not necessarily preclude reproductive development. That is, the vegetative and reproductive states may occur simultaneously, at least in the strawberry, provided the environmental conditions are favorable.

Summary

1. Missionary strawberry plants were grown under controlled-environment conditions, with the temperature and day-length regulated, for the purpose of studying the effect of these factors on the vegetative and reproductive responses of this plant.

2. Strawberry plants showed about the same positive reproductive response to short photoperiods whether they were grown at constant temperature or at daily fluctuating temperature.

3. A 20-hour photoperiodic cycle (10 hours light and 10 hours darkness) failed to result in runners. A 28-hour photoperiodic cycle (14 hours light and 14 hours darkness) did cause runner production.

4. Flowers were formed under both the 20- and the 28-hour photoperiodic cycle.

5. Photoperiodic induction was found to occur in the strawberry. For the Missionary variety, exposure to seven short photoperiods resulted in flower formation although the plants were subsequently removed to long days. Exposure to three short photoperiods failed to result in floral development.

6. Flower formation occurred in strawberry plants held under long days provided one or more leaves were subjected to short photoperiods. Runner

plants growing in long days were induced to flower, provided they remained attached by a runner to parent plants growing in short days.

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CAROTENOID PIGMENTS IN TUBERS OF THE KATAHDIN VARIETY OF IRISH POTATO

BYRON C. BRUNSTETTER AND HERBERT G. WISEMAN

(WITH ONE FIGURE)

Received March 20, 1947

Introduction

Flesh color in Irish potato varieties ranges from almost pure white to a deep canary yellow. The yellowish tinge of "white-fleshed" varieties is intensified after dehydration, becoming a definite yellow if the tubers have been harvested in an immature state, as is the case with material grown in the south (**1, 2**).

This residual yellow color, when it occurred, was at one time considered by the Army Quartermaster Corps to be due to a faulty technique in dehydration and as a result such material was graded downward. It was soon recognized, however, that this yellow color was due to the presence of naturally occurring carotenoid pigments. CALDWELL, BRUNSTETTER, CULPEPPER, and EZELL (**3**) showed that 19 "white-fleshed" varieties, representing fully-matured material, averaged 0.021 mg. of total carotenoid pigments per 100 gm. fresh weight, while 3 normally yellow-fleshed varieties averaged 0.138 mg. per 100 gm. fresh weight. This means that when the total concentration of carotenoid pigments is about 1 p.p.m. on a dry weight basis, the yellow color of the dried product will be slight. When the total pigment concentration is about 7 p.p.m. dry weight, the dried product will have a marked yellow color. One of the findings of the present paper is that in the case of the Katahdin variety, harvested in an immature state, there was still a pronounced yellow color in the dried product when the concentration of carotenoid pigments was 3 p.p.m. dry weight.

This paper reports an intensive study of the carotenoid pigments in three samples of the Katahdin variety, with the purpose of separating and identifying as many of the pigments as possible.

Review of literature

Critical work on the number and nature of carotenoid pigments present in Irish potatoes is very scanty. SCHMID and LANG (**13**) analyzed yellow internal regions of the Kipfler variety in connection with the so-called "yellow-fleck" disease. From the saponified epiphase fraction they isolated a xanthophyll pigment which was purified by adsorption on a $CaCO_3$ column. Its spectral absorption curve had maxima at 4380 and 4660 A, so that they identified the pigment as "beta xanthophyll." A second pigment, isolated from the hypophase fraction by adsorption on $CaCO_3$, had absorption maxima at 4435 and 4725 A, which as the authors noted, are

characteristic both of violaxanthin and taraxanthin. Incidentally, these absorption maxima are given by ZECHMEISTER and TUZSON (**16**) for the beta isomer of luteol. A third pigment isolated from the epiphase fraction by adsorption on Al_2O_3 showed broad absorption maxima at 4490 and 4770 A and according to the authors resembled the absorption spectrum of alpha-carotene.

EULER, AHLSTRÖM, HÖGBERG, and TINGSTAM (**4**) in a nutritional study of the value of the carotenoid pigments from steamed samples of a number of yellow-fleshed varieties of potatoes grown in northern Sweden, separated carotene by chromatographic adsorption on a column of Brockman's aluminium oxide. The total carotenoid pigments ranged from 0.77 to 2.58 p.p.m. fresh weight, with the carotene content varying from 0.066 to 0.10 parts per million.[1]

Methods and materials

Three grades of petroleum ether, referred to as Sk F, Sk B, and Sk C, were purified by stirring successively with concentrated H_2SO_4, acid dichromate solution, NaOH, and four changes of distilled water. After stirring with, then filtering through, silica gel the lots of petroleum ether were distilled over solid KOH. Industrial absolute ethanol was used without purification.

Regarding the chromatographic apparatus, two details of design should be noted: (1) the tube in which the adsorbent was packed had a sintered glass filter at the bottom; (2) the tube was jacketed to provide cooling by continuous circulation of absolute ethanol refrigerated by a mixture of solid carbon dioxide and ethanol. This insured chromatographic analyses at a temperature between $-50°$ and $-60°$ F. The adsorbent suspended in Sk F was packed in the chromatographic column by pressure from a tank of nitrogen. After introduction of the sample, the solvent necessary for the development and elution of the various carotenoid zones was forced through the column by this source of pressure. These zones were caught in separate receivers.

An advantage of a refrigerated column is that it permits the use of Sk F whose low boiling point facilitates evaporation in vacuo at room temperature. Since the force of adsorption increases as the temperature is decreased, adsorbents such as magnesium carbonate, the decahydrate of sodium sulphate, or sucrose, which adsorb so feebly at room temperature as to be quite unsatisfactory, perform excellently at low temperatures such as $-50°$ F. Finally chromatographic analyses at low temperatures retard changes such as formation of stereoisomers, or oxidation of unstable carotenoids.

Absorption data were obtained by a photoelectric spectrophotometer which has a Hilger double monochromator with quartz optics. The slit

[1] The figures referring to the results of EULER and associates as given in reference (3) should be divided by 100.

width generally used was 0.06 mm. giving a spectral isolation of one millimicron for the mercury line located at 4358.3 A, which was used for the routine calibration of the wave length scale.

The data are expressed in terms of optical density which is equal to the difference between the log incident light (I_o) and the log transmitted light (I). The density is the product of (a) the specific absorption coefficient, (b) the concentration in grams per liter of solution, and (c) the cell length in centimeters.

The first sample consisted of Katahdin potatoes that had been grown in Maine during the season of 1943. Normal maturing of the tubers was retarded by a cold rainy autumn; in fact the vines had to be killed by spraying with chemicals in order to harvest the crop. Accordingly the amount of carotenoid pigments was sufficiently great to impart a strong yellow color to the dried product. Before dehydration, the strips of potatoes were treated with sulphur dioxide to prevent heat-reddening.

The second lot of Katahdin potatoes was grown in Baton Rouge, Louisiana, in the spring of 1944. It was also relatively immature so that the dried product had a yellow color. Undried potatoes (Sample II) were analyzed as well as potatoes treated with sulphur dioxide after steam blanching and before drying (Sample III).

The preparation of the crude carotenoid solutions, essentially similar for the three samples, is described in the following steps:

1. The samples were extracted with absolute ethanol.

Sample	Grown in	Condition	Dry weight	Ground in ball mill with ethanol
I	Maine	Undried	1303 gm.	as finely ground powder
II	Louisiana	Dried	1000 "	as slices previously extracted with ethanol
III	Louisiana	Dried	1000 "	as Julienne strips

After the undried sample was washed, the skin was removed by vigorous rubbing with towels. The sliced sample weighing 8056 gm. was dehydrated and partially extracted with ethanol at about 10° C. for 6 days. After decanting the extract, the residue was ground under ethanol in a ball mill.

2. The alcoholic extracts were filtered and transferred to Sk F by the addition of excess distilled water, concentrated in vacuo, and washed free of residual ethanol. In the removal of these pigments from the ethanol extract, NaCl was used to break up emulsions and to reduce the solubility of hydroxylated carotenoids in ethanol. Care was taken to remove all the pigments from the ethanol extract.

3. The carotenoids were then partitioned between Sk F and 92% methanol. The upper layer (epiphase) was freed from methanol by washing with water, concentrated in vacuo, and made up to volume. The lower layer (hypophase) was transferred to Sk F by the addition of excess NaCl. At this point also the danger of incomplete extraction of hypophasic pigments was recognized and avoided as much as possible. The petroleum

ether phase was then washed with water to remove methanol, concentrated in vacuo, and made up to volume.

Heating in order to concentrate extracts was avoided throughout all operations in order to prevent acceleration of reversible stereoisomeric changes or processes of decomposition.

Experimental results

A 10-ml. aliquot from the hypophase stock solution (50 ml.) of Sample I was chromatographed on a column of confectioners' sugar containing 3% starch. Eight zones were developed and eluted into separate containers. Spectrophotometric readings of the corresponding solutions made at wave lengths of 4000, 4450, and 4700 A amounted to 93, 88, and 102%, respectively, of the readings of the unchromatographed solution measured at the same wave lengths. Here, as throughout this work, development and elution of the various zones were obtained by the successive use of solutions of ethanol in petroleum ether, with a low initial concentration (0.05%) which was increased by small amounts, such as from 0.1 to 0.2%, to an upper concentration determined by the intensity with which the top zone was adsorbed. The judicious use of weak and increasingly concentrated solutions of ethanol in petroleum ether has often been of decided aid in the separation of fractions whose forces of adsorption were nearly alike.

The volume of hypophase stock solution of Sample II was 100 ml. An aliquot (10 ml.) was sent through a column of $MgCO_3$ on top of which was a cap of confectioners' sugar. A fraction containing colorless impurities was readily separated. The remaining pigments were rapidly eluted with 3% ethanol in Sk F, evaporated to dryness, and redissolved in a small volume of Sk F. This solution was then fractionated on successive columns of confectioners' sugar. Twelve zones were developed and collected separately.

However, the separation of the components of the hypophase fraction from Sample III presented difficulties. Adsorption on a column of confectioners' sugar alone was so great that effective development of different zones was impossible. Excellent separation was obtained on a column of Na_2SO_4 alone and mixed in various proportions with confectioners' sugar. Thirteen components were developed by elution but in spite of the fact that the column was cooled to a low temperature, only 54% recovery, measured at 4400 A, was obtained. This loss was largely avoided by the use of $Na_2SO_4 \cdot 10H_2O$, alone and as a diluent of confectioners' sugar. From a 10-ml. aliquot of a 100-ml. stock solution 21 components, most of them minor in amount, were developed and eluted separately and a recovery of 88% measured at 4400 A, was obtained.

For the benefit of workers who may desire to use this last absorbent, a few remarks on technique may be helpful. After preparation of $Na_2SO_4 \cdot 10H_2O$, it was stored under 95% ethanol. In preparation for a run a portion was placed on a Buchner funnel and washed free of ethanol

solution by the use of petroleum ether. The salt was then suspended in Sk F and stirred several minutes in a Waring Blendor. The absence of free water is essential because if present in a refrigerated column it will freeze so that elution becomes excessively slow.

Toward the end of this investigation, preliminary stirring in a Waring Blendor of any adsorbents suspended in Sk F was adopted as a routine procedure, since it greatly facilitated uniform packing of a column, a condition necessary for difficult separations.

In every one of the hypophase fractions of the three samples the first zone to be eluted had essentially the same spectral absorption characteristics. While maxima and minima were lacking in the visible region of the spectrum, there were large and rapid increases in densities as the wave length was decreased to 3400 A, the lower limit of our measurements. The absorption curves for these bottom zones were similar to an absorption curve published by McNicholas (**10**) for a solution of lutein stored 164 days. These zones contained a relatively large amount of colorless impurities, easily soluble in petroleum ether and difficultly soluble in ethanol. The impurities could be readily removed by lowering the temperature of the ethanol solution in a freezing mixture and then decanting. Experiment showed that they were not responsible for the marked absorption in the near ultraviolet region.

Spectrophotometric data on hypophase sub-fractions of the three samples are given in table I. The solvent for the fractions in Sample I was Sk B; for the fractions in Sample II and III, the solvent was ethanol. The wave lengths of the maxima, minima, and shelves or plateaus are tabulated together with their relative densities (R.D.) based on the densities of the principal maxima. The fractions or zones are numbered according to their relative adsorbability. The smaller the number, the less was the adsorbability of the pigment and the nearer its position to the bottom of the column. In Sample III, a number of chromatographic analyses were made involving different columns; the numbers here denote relative ease of adsorption. Fractions omitted fall into two classes: (a) those that appeared to be mixtures and (b) those with concentrations so low that only a few readings could be obtained.

Lutein

Fraction 4, Sample II (with ethanol as a solvent) showed good agreement in details of absorption with the curve published by Zscheile, White, Beadle, and Roach (**18**) with the exception that the maximum at 4775 A and the minimum at 4625 A were displaced slightly toward the ultraviolet.

Fraction 3, Sample I also was lutein. The absorption curve indicates that in this case stereoisomers of lutein were present. According to Zechmeister and Tuzson (**16**) lutein, like other carotenoids, can be reversibly isomerized. They found two isomers in approximately equal amounts comprising about 40% of the initial material. Dissolved in ethanol, neolutein

TABLE I

SPECTROPHOTOMETRIC DATA ON CAROTENOID PIGMENTS (HYPOPHASE FRACTIONS) FROM IRISH POTATO TUBERS

FRACTION	MAXIMA	R.D.*	MINIMA	R.D.	SHELVES	R.D.
Sample I	*A*		*A*			*A*
3	4430–4460	100	4600–4620	71	.....	.....
	4720–4730	89	4260–4270	69	....	.....
	4200–4220	69	.....	.....	.....	.....
4	4460–4490	100	4330–4350	93	4000–4100	68
	4270	95	.....	.....	.....	.....
5	4420	100	4580	73	4200–4250	77
	4680–4690	83	.....	.....	.....	.....
6	4200	100	4050	69	.....	.....
	4480	94	4350	69	.....	.....
	3975	76	.....	.....	.....	.....
7	4480	100	4350	71	4600–4750	42
	4200–4230	97	.....	.....	.....	.....
8	4260	100	4120	74	4420–4470	63
	4010	93	.....	.....	.....	.....
Sample II						
4	4460	100	4610	73	.....	.....
	4750	90	4250–4260	70	.....	.....
	4230–4240	70	.....	.....	.....	.....
5	4460	100	4610	71	4200–4250	68
	4720–4740	91	.....	.....	.....	.....
7	4250	100	4400–4430	81	4000–4100	78
	4500	87	.....	.....	.....	.....
9	4445	100	4620	72	.....	.....
	4725	90	4275	70	.....	.....
	4225	71	.....	.....	.....	.....
10	4400–4450	100	4600	77	.....	.....
	4650–4700	82	.....	.....	.....	.....
12	4260	100	4125	74	.....	.....
	4025	83	.....	.....	.....	.....
Sample III						
6	4425–4450	100	4600–4625	77	4200–4250	73
	4700–4750	87	.....	.....	.....	.....
8	4400–4425	100	4600	76	4180–4250	75
	4680–4700	85	.....	.....	4200–4250	84
11	4420	100	.....	.....	4200–4250	84
	.....	.....	.....	.....	4600–4700	86
14	4450–4480	100	4600–4625	76	4200–4250	70
	4725–4760	90	.....	.....	.....	.....
16	4400–4500	100	4625	80	4550–4600	83
	4700	91	.....	.....	.....	.....
17	4425–4475	100	.....	.....	4250–4300	91
	.....	.....	.....	.....	4675–4700	90
18	4400	100	4600	78	4200–4250	80
	4650	81	.....	.....	.....	.....
19	4450–4500	100	4350	94	4250–4300	96
	.....	.....	.....	.....	4700–4725	70
20	4400–4475	100	4625	80	4250–4300	87
	4700	80	.....	.....	.....	.....
21	4440–4460	100	4350	92	4625–4700	65
	4200–4220	99	....	.....	.....	.....

* Relative density based on the densities of the principal maxima.

A gave absorption maxima at 4710 and 4420 A; the corresponding maxima for neolutein B occurred at 4720 and 4430 A. The broad absorption maxima of Fraction 3, Sample I was evidence for the presence of stereoisomers.

Further evidence was the presence of a slight peak at 3300 A, for ZECHMEISTER and POLGÁR (17) state that lutein in equilibrium with its two isomers shows a maximum (the "cis peak") at 3310 A (hexane).

The concentration of lutein in parts per million dry weight was 0.50 in Sample I, and 0.77 in Sample II.

In the processing of Sample III, the strips of potato tissue were steam-blanched for 7 minutes, then placed immediately in a sulphurous acid solution (0.25%) for 30 seconds. As a result the dried product had a pronounced taste, and the initial alcoholic extract had a pronounced odor of sulphur dioxide. As previously stated, the hypophasic pigments with the exception of the first fraction were adsorbed on a column of confectioners' sugar far more strongly than the hypophasic pigments of the other two samples. This pronounced increase in adsorption may be interpreted as due to an increase in the number of hydroxyl groups in the various carotenoid pigments of this sample, a chemical change which need not necessarily be accompanied by more than slight changes in the absorption spectra. These considerations, along with the presence of a shelf instead of a maximum and a minimum in the region 4200–4260 A, appear to rule out Fraction 6, the first major hypophasic fraction of Sample III, as lutein.

Fraction 5, Sample II, was adsorbed immediately above lutein and its absorption curve was closely similar to lutein. The chief difference was the absence of a maximum and a minimum in the region 4200–4260 A. Consequently, this fraction is tentatively identified as isolutein.

FLAVOXANTHIN

This pigment, it will be recalled, was first isolated by KUHN and BROCKMANN (9) from *Ranunculus acer* (buttercup). KARRER and JUCKER (5) showed that its molecule has two hydroxyl groups, and that the third O atom is present in an ether-like bond. STRAIN (14) demonstrated its presence in green leaves and showed that it is adsorbed above lutein on a magnesia column. STRAIN's measurements of its absorption curve in ethanol solution located the maxima of the two flavoxanthin isomers at 4220 and 4520 A. Among the seven xanthophylls which STRAIN isolated from green leaves, flavoxanthin alone has absorption maxima at these positions.

According to the absorption data in table I for Fraction 6, Sample I, this fraction was a flavoxanthin. Fraction 7, Sample II, does not check well with STRAIN's data, and it is probable that this fraction contained impurities which rechromatographing would have removed. A third fraction, 21 from Sample III, also possessed maxima whose positions approximated those of flavoxanthin. However, in view of its relative adsorbability and the lack of agreement in details with the spectral adsorption curve of flavoxanthin, it is improbable that this fraction contained flavoxanthin.

AUROXANTHIN

This pigment, isolated and described by KARRER and RUTSCHMANN (7, 8), is distinguished by sharp absorption maxima of equal intensity

located at 4030 and 4280 A. Absorption maxima of lesser intensities are located at 3820 and 2940 A.

The absorption data for Fraction 8, Sample I, and Fraction 12, Sample II, had very sharp absorption maxima which checked closely with the two principal absorption peaks of auroxanthin. As in the case of auroxanthin, inflections between 4400 and 4500 A were present. No examination of the absorption was made in the region below 3500 A. In our preparation there were shelves instead of peaks in the region 3800–3900 A. Furthermore, the densities at 4280 and 4030 A were unequal. Further work on a more highly purified preparation would be necessary to establish the identity between this pigment from potato tubers and auroxanthin.

The remainder of the hypophasic pigments listed in table I could not be identified.

Epiphase fractions

The epiphase portion of Sample I was dissolved in Sk F and adjusted to a volume of 50 ml. For chromatographic analysis, an aliquot of 5 ml. was poured on a column of $MgCO_3$ on top of which was a layer of confectioners' sugar. Elution with Sk F alone separated the yellow bottom zone which was the main fraction. Six other zones were developed and separated by elution with solutions of ethanol in Sk F, starting with a 0.1% solution and going by steps to a 3% solution. The third zone from the bottom was further fractionated into a blue zone (which later in the course of elution changed to a green color) with a yellow zone immediately above it. The fourth zone from the bottom was green in color. On top of the layer of sugar there was a blue zone which could not be eluted with a 3% ethanol solution. These interesting zones were not examined spectrophotometrically.

The bottom zone (fraction 1) was rechromatographed on the same type of column. A major bottom zone (fraction 1–1) with two yellow zones adsorbed above it were separated. Absorption curves of these upper zones showed no maxima or minima; both fractions showed a continuous increase in density with sharp increases in rate at 3800–3700 A. The curves were similar to the absorption curves of the first zones eluted from the hypophase fractions.

In the course of this work on the epiphase solutions, commercial beta-carotene was purified by several recrystallizations from Sk F by additions of ethanol and its absorption spectrum was measured. The data will be given here for comparison with the carotene fractions listed in table II: 4525 A, density 100; 4805 A, 87.90; 4700 A, 81.0; 4300 A, density 70.7.

As shown in table II, Fraction 1–1 from Sample I checked fairly well with beta-carotene as regards relative densities; though, as might be expected from unrecrystallized material, there was a displacement of the minimum and the principal maximum toward the ultraviolet. At wave lengths below 400 A the density increased to 3000 A, parallel to that of the other two fractions. The pigment was unstable, for the densities of the principal maxima changed during measurement. This might have been predicted for

(a) the sample had been ground to a fine powder and (b) the original ethanol extract had been stored for about 3 weeks at −40° C.

The concentration of Fraction 1–1, calculated as carotene, was 0.38 p.p.m. dry weight.

The epiphase solution of Sample II also was a complex mixture of carotenoid pigments. An aliquot of 5 ml. from a stock solution of 50 ml. was chromatographed on a column of $MgCO_3$. At least nine zones were developed, six of which were obtained separately by elution. The third zone from the bottom was a composite of an orange zone, two yellow zones, and a greenish zone. The sum of the densities measured at 4500 A was 94.6% of the unchromatographed epiphase solution, evidence of minor loss only.

As in the case of Sample I, the bottom zone was the main fraction; measured at 4500 A its density was 42% of that of the total carotenoids in the epiphase solution and 9% of the total carotenoids in both hypophase and epiphase solutions. Calculated as carotene, this fraction was present in a concentration of 0.31 p.p.m., dry weight.

Referring to the spectrophotometric details given under 1, Sample II, in table II, the broad absorption maximum at 4485–4520 A, the shift in position of maxima and minima toward the ultraviolet and the presence of a shelf in the curve at 4250–4300 A indicate that this fraction was a mixture of stereoisomers. The relative densities checked with those of all-trans-beta-carotene with the exception that the curve in the region of 4300 A was about 10% too high.

Fraction 2, adsorbed immediately above fraction 1, was particularly interesting since the relatively high density from 4270 to 4330 A indicates a carotenoid component with an absorption maximum in this region. This point will be reexamined later.

Analyses of the absorption curves of the other zones in Sample II indicated that they too were mixtures, a peculiarity of the third (composite) zone from the bottom being the presence of a pronounced absorption peak at 4120 A.

This run confirmed the results of the analysis of the epiphase fraction of Sample I in showing that a column of $MgCO_3$ even when cooled below −50° C. was not efficient in separating epiphasic pigments, at least in a single chromatography. Very probably repeated chromatography would have given a better separation. The results clearly show, however, that in the absence of factors such as treatment with SO_2 and drying, a complex mixture of pigments is obtained not only in the hypophase, but also in the epiphase fraction.

When a 10-ml. aliquot of the epiphase fraction of Sample III (100 ml. Sk F) was chromatographed on $MgCO_3$ spectrophotometric measurements of the bottom (main) fraction (table II, Sample III; Run 1–a) showed at once that the relative density at 4275 A was much higher than was the case in the other two samples. This high ratio was present also in the case of the other three upper zones. Rechromatographing this first fraction on a

column with a bottom layer of confectioners' sugar, a main layer of $Ca(OH)_2$, and a top layer of $MgCO_3$, did not change essentially the relative density at 4275 A. Calculated in terms of carotene, this first fraction was present in a concentration of 0.61 mg. per kg. dry weight, almost twice the values obtained for the "carotene" fractions of the first two samples, evidence that it was impure. This bottom zone had a density at 4400 A that was 55% of the total epiphasic density.

The separation was repeated using a 30-ml. aliquot and chromatographing twice on columns of $MgCO_3$, a middle layer of equal parts of $MgCO_3$ and $Ca(OH)_2$, then a bottom layer of confectioners' sugar. Five zones were separated. The ratios of the density values at 4300 and 4500 A were 75 and 238% for the bottom and top zones respectively, showing definitely that both the main fraction in the previous run and the zones adsorbed above it had been contaminated with a pigment absorbing strongly near 4300 A.

The top zone was then chromatographed three times on a column of 2 parts of $Ca(OH)_2$ to 1 part of $MgCO_3$. The final product was characterized by a density at 4300 A that was 626% of the density at 4500 A. Detailed spectrophotometric analysis of this fraction dissolved in ethanol showed the characteristics listed in table II, Sample III, Run 1–b, Fraction 1–a–5–c–2.

In spite of repeated chromatographic analyses, this yellow fraction contained a lower pink zone in relatively small amount which could not be eluted separately. Apparently it did not interfere with the absorption of the yellow zone as the peaks and minima of the latter were very sharp, and corresponded closely with values for a carotenoid given the provisional name of ξ-carotene by STRAIN and MANNING (15). This carotene has been found in carrots, yellow corn, and in certain strains of tomatoes. A review of the literature has been given recently by NASH and ZSCHEILE (11) who have measured its absorption spectrum and present specific absorption coefficients. They state that ξ-carotene exhibits typical isomerisation phenomena and suggest that it may occur in nature in a wide variety of sources. Recently PORTER, NASH, ZSCHEILE, and QUACKENBUSH (12) have found that ξ-carotene is biologically inactive as provitamin A.

To check on the carotene nature of sub-fraction 1–a–5–c–2 (ξ-carotene) it was dissolved in Sk F and shaken for one hour at room temperature with 20 ml. of a saturated solution of KOH in methanol. Subsequent partition by shaking with methanol showed that no pigment transferred to the methanol layer, and that therefore ξ-carotene is not an esterified carotenol. It was then transferred to ethanol, made up to volume, and remeasured after a month's storage at $-4°$ C. A marked general decrease in density was noted. A slight maximum at 3550–3600 A and a slight minimum at 3600 A were observed.

The presence of ξ-carotene was confirmed by a second preparation (Run 2). A subsample of III weighing 334 gm. was extracted by grinding overnight in the ball mill in the presence of two grams of $CaCO_3$ using specially purified absolute ethanol. An aliquot of the filtrate equivalent to 230 gm.

of sample was transferred to Sk F, concentrated to a volume of one liter, then shaken twice in two 500-ml. portions with successive charges of 25 ml. of methanol saturated with KOH. The pigments were partitioned in the usual manner between 92% methanol and Sk F and the two fractions obtained were then transferred to Sk B.

Twenty-five ml. of the epiphase fraction (100 ml.) were chromatographed on a column composed of 40% of $Ca(OH)_2$ and 60% of $MgCO_3$. This separation was unsuccessful; so the pigments were eluted with 4%

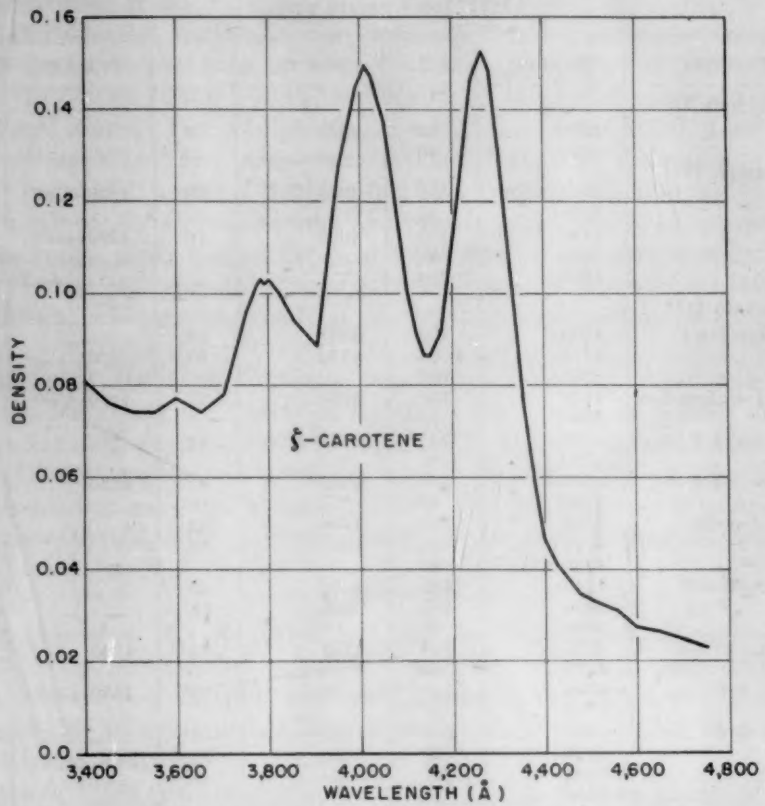

FIG. 1. Absorption spectrum of ζ-carotene, separated from crude carotene of epiphase solution, from tubers of Katahdin variety of Irish potatoes.

ethanol in Sk F, evaporated to dryness in vacuo, and redissolved in Sk B. The second column was composed of $MgCO_3$ with a top layer of confectioners' sugar. The bottom zone obtained from this column was rechromatographed on a column of 20% $Ca(OH)_2$ and 80% $MgCO_3$. A yellow bottom zone was eluted with Sk F alone (see table II, Run 2, 2–a; this pigment could not be identified). The zone above it was composed of two sub-fractions (the lower, pink in color; the upper, yellow) which could not be separated sufficiently to be collected separately. The next zone was minor in

amount. At the top of the column there was a yellow zone which was eluted with 0.5% ethanol in Sk F and collected separately. The details of the absorption curve (see table II, Sample III, Run 2–d; compare with 1–a–5–c–2) checked with those of the previous run and showed moreover a slight peak at 3600 A. This curve is reproduced in figure 1. In this run the amount of ξ-carotene obtained was 134% of the amount obtained in the

TABLE II

SPECTROPHOTOMETRIC DATA ON CAROTENOID PIGMENTS (EPIPHASE FRACTIONS) FROM IRISH POTATO TUBERS

FRACTION	MAXIMA	R.D.	MIMIMA	R.D.	SHELVES	R.D.
Sample I‡	*A*		*A*			
1–1	4500	100	4650	79	.....	.....
	4800	90	.....	.....	.....	.....
Sample II‡						
1	4485–4520	100	4660	82	4250–4300	78
	4760	89	.....	.....	.....	.....
2	4445	100	4630	76	4270–4330	89
	4720	81	.....	.....	.....	.....
	4200	90	.....	.....	.....	.....
Sample III*						
Run 1–a 1	4500	100	4687	84	.....	.....
	4775	86	4350	85	.....	.....
	4275	94	.....	.....	.....	.....
Run 1–b: 1–a–1–a	4450	100	4600	77	4200–4500	73
	4760	89*	.....	.....	.....	.....
1–a–1–b	4500	100	4725–4750	84	.....	.....
	4725	85	.....	.....	.....	.....
1–a–3–4–b–3	4475	100	4650	82	4250	73
	4725	86	.....	.....	.....	.....
1–a–5a	4500	100	4700	82	.....	.....
	4750	85	4340	83	.....	.....
	4250–4275	86	.....	.....	.....	.....
1–a–5–c–2	4240	100	4150	53	.....	.....
	4000	99	3880	56	.....	.....
	3800	69	.....	.....	.....	.....
Run 2: 2–a	4525	100	4700	85	4200	70†
	4775	86	.....	.....	.....	.....
2–b	4500	100	4700	83	4300–4350	80
	4750	86	.....	.....	.....	.....
2–d	4260	100	4150	56	.....	.....
	4010	98	3900	58	.....	.....
	3800	67	3650	48	.....	.....
	3600	50	.....	.....	.....	.....

* Estimated by extrapolation.
† Inflection.
‡ Solvents: Sample I, Sk B; Samples II and III, ethanol.

previous preparation. The density at 4250 A was 12.5% of the entire epiphase fraction. Using the value of 226 for the specific absorption coefficient of ξ-carotene at 4260 A (NASH and ZSCHEILE), the concentration of ξ-carotene was calculated to be 0.11 p.p.m. dry weight.

Returning now to the description of the remaining four zones of Run 1–b, the bottom zone, 1–a–1, was rechromatographed on a column whose top and bottom layers were confectioners' sugar and whose main part con-

sisted of a mixture of 2 parts of $CaOH_2$ and one part of $MgCO_3$. Two subfractions, 1–a–1–a and 1–a–1–b, were separated. It is apparent from the spectrophotometric data in Table II that neither of them was trans-beta-carotene and that probably they consisted in whole or in part of cis-trans stereoisomers. The ratio of densities at 4300, 4500 and 4800 A for the two fractions above the bottom zone checked with the corresponding ratios for pure trans-beta-carotene. Calculating their sum as carotene the concentration was 0.19 p.p.m. dry weight, about one-third the concentration of the crude carotene fraction.

Unfortunately complete curves were not obtained for these fractions or for the fourth zone from the bottom. Instead the third and fourth zones from the bottom of the column were combined and rechromatographed on a column consisting of a sugar base, a middle layer of $Ca(OH)_2$ and one part of $MgCO_3$. Three zones were obtained. Data on the top zone are listed in table II under 1–a–3–4–b–3. This fraction dissolved in ethanol was stored at −4° C. for one week and then remeasured. No displacement of absorption peaks had occurred, and the density was only slightly less. This fraction corresponded closely as regards location of peaks and relative densities to neo-beta-carotene-U as described by ZECHMEISTER and POLGÁR (17).

As noted above, the top zone was chromatographed three times on columns consisting of 2 parts of $Ca(OH)_2$ and 1 part of $MgCO_3$ in the separation and purification of ξ-carotene. A fraction obtained after the first chromatographic separation was further resolved and studied in detail. Pertinent data are given in table II under fraction 1–a–5–a. The absorption curve indicated that an unidentified cis type of carotenoid was present.

Discussion

In this work, in spite of the fact that the total amount of carotenoids available for investigation was only about 3 mg., at least a dozen different zones representing different carotenoid pigments were developed on chromatographic columns though not all these could be separated in a form suitable for analysis.

Among them perhaps the most interesting were the two pigments with sharp major absorption peaks around 4000 and 4250 A. One of these was in the hypophase portion and was located at the top of columns of confectioners' sugar. As already noted, the location of the two major absorption peaks agrees fairly closely with those reported by KARRER and RUTSCHMANN (7) for auroxanthin. Their method of separation emphasizes the tenacity with which this pigment is adsorbed, for they employed a column of zinc carbonate, a mild adsorbent, and used for elution a mixture of 1 part of methanol to 2 parts of ether. Their source of material was the flower, *Viola tricolor*.

From KARRER's laboratory a series of recent reports have clarified the relation between major carotenoids and their oxidation products. In gen-

eral, a carotenoid lacking oxygen or containing oxygen only in the form of hydroxyl groups is treated with monoperphthalic acid. This results in an epoxy compound, the oxygen introduced bridging two adjacent carbon atoms in the β-ionone ring. These epoxy derivatives are very sensitive to the action of acids. For example, the minute amount of HCl in "aged" chloroform will rapidly transform dissolved epoxy carotenoids to furanoid oxide isomers. As a result marked displacements of the absorption maxima toward the ultraviolet occur. Specifically KARRER and JUCKER (**5**) found that lutein epoxide isomerized to form two carotenoids which were identical with flavoxanthin and chrysanthemaxanthin, each containing two –OH groups with the third oxygen atom in an ether-like union. Zeaxanthol has two cyclohexenyl rings and so forms a mono-epoxide and a di-epoxide which were found to be identical with antheraxanthin and violaxanthin respectively. Treated with acid, antheraxanthin isomerized to mutatoxanthin, while violaxanthin isomerized to auroxanthin. The acid treatment split off oxygen to some extent from violaxanthin so that mutatoxanthin and the starting material, zeaxanthin, were also present.

They further found by the same technique (**6**) that β-carotene also forms a mono-epoxide and a di-epoxide. Treated with acid the former isomerized to mutatochrome and the latter to aurochrome.

Thus from known carotenoids easily obtained in relatively large amounts, other carotenoids, most of them previously isolated from natural sources in small amounts, were prepared by in vitro chemical reactions. KARRER and JUCKER believe that the in vitro method of preparation essentially parallels the processes in the plant which produce these less abundant carotenoids.

It follows then that the detection of auroxanthin in Sample II (undried) implies the presence of violaxanthin and zeaxanthin. These two carotenoids may have been among the fractions studied, but they could not be detected with certainty, probably because the impurities or cis stereoisomers present obscured the none too distinctive absorption spectra. The absorption spectrum of auroxanthin is quite characteristic distinguishing it sharply from all other hypophasic carotenoid pigments, so that its presence in Samples I and II seems to be fairly certain.

It is possible that auroxanthin may be widely distributed and that the reason it has not been generally noted is the fact that only a mild adsorbent with an eluent containing a polar solvent serves to separate it. One would expect it to be held in an uppermost zone on a column such as magnesium oxide. In this connection it is of interest that STRAIN (**14**) in his monograph on leaf xanthophylls records 11 zones separated on columns of magnesia. The topmost three zones, orange-yellow in color, are denoted as mixtures with two absorption maxima, one at 4500 A and an "indefinite" maximum at a shorter wave length. The authors suggest that one or more auroxanthin-like compounds may be separated by rechromatographing these upper three zones on a column of either confectioners' sugar or on $Na_2SO_4 \cdot 10H_2O$.

The second pigment of this type was ξ-carotene, appearing in the crude carotene fraction of Sample III. Unfortunately, due mainly to the fact that the aliquots taken for chromatographic analyses were too small, its presence in the carotene fractions of the other two samples could not be definitely established. Another factor which very probably was largely responsible for its apparent absence was the fact that in Sample II the epiphase had been stored in ethanol for 20 days. As STRAIN (**15**) and NASH and ZSCHEILE (**11**) have observed, ξ-carotene rapidly oxidizes in polar solvents. Unfortunately this fact was not recognized in the present work so that the concentration of ξ-carotene reported for Sample II is very probably too low. Furthermore, Sample I had been ground to a fine powder so that ξ-carotene, if present, would have been exposed to conditions making for maximum oxidation. In the case of Sample III, only a week elapsed from the extraction of the sample to the storage of the epiphase in Sk B.

KARRER and JUCKER (**6**) state that the absorption spectra of auroxanthin and aurochrome are practically identical. Similarly there is a close agreement between the absorption maximum of auroxanthin and that of ξ-carotene. Aurochrome is adsorbed above β-carotene on a column of $ZnCO_3$. Similarly, ξ-carotene is adsorbed above β-carotene on a suitable column. In view of these facts the authors suggest that ξ-carotene is identical with aurochrome and therefore is derived from β-carotene by oxidation to the di-epoxide followed by isomerisation to the furanoid oxide form thus reducing the number of conjugated double bonds and displacing the positions of the absorption maxima toward the ultraviolet. If the two pigments are identical then "ξ-carotene" like aurochrome should, when its ethereal solution is shaken with 25% HCl, show a stable deep blue color. Whether ξ-carotene does so react with concentrated HCl is unknown at present.

Although the total amount of carotenoid pigments present in the undried sample of Katahdin potatoes (II) was approximately 3 mg. per kg. dry weight, it was sufficient to give the dried product a marked yellow color. It is interesting to note that dehydration of this sample lowered the total carotenoid content only 10%.

The concentration of beta-carotene was about 10% of the total carotenoids present and obviously it was present in such small amounts as to be negligible from a nutritional point of view.

Summary

1. The Katahdin variety of potato, when harvested in a relatively immature condition, contains a complex mixture of carotenoid pigments. The concentration of total pigments was about 3 parts per million, dry weight, a concentration sufficient to impart a yellow color to the dehydrated product.

2. Lutein was found in two of the three samples studied, in concentrations of 0.50 and 0.77 parts per million, dry weight. A pigment corresponding to isolutein was present in one sample.

3. A flavoxanthin was found in one sample and tentatively identified in a second sample.

4. A carotenoid provisionally identified as auroxanthin was present in two samples.

5. An epiphasic pigment with sharp absorption maxima located at 3800, 4000, and 4250 A was separated from one sample. It was identical with ξ-carotene. Its concentration in potatoes was at least 0.11 parts per million, dry weight. It is suggested that ξ-carotene is identical with aurochrome, derived by KARRER and JUCKER from β-carotene.

6. Beta-carotene was detected; its concentration was about 0.3 parts per million, dry weight. In one of the samples, the presence of the stereoisomer, neo-beta-carotene-U was detected.

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THE SUGARS OF THE ROOTS OF *DAUCUS*[1] *CAROTA*

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It is well known that some reducing substances, resembling true sugars in their behavior, occur almost invariably in plant extracts and may introduce great errors into the estimation of those sugars. Since the soluble sugars commonly present in plants (glucose, fructose, sucrose and maltose) are easily fermented by yeast, this phenomenon suggests itself as a convenient and practicable method for the fractionation of the total reducing substances in any plant extract into fermentable and non-fermentable fractions.

Methods

TRIALS ON THE FERMENTATION OF PURE SUGARS BY ORDINARY BAKER'S YEAST

After trials with various brands of baker's yeast, a commercial brand, known in England by the name "Eureka" was selected as being most suitable.

Considerable work was done to determine optimum conditions for fermentation of pure sugars, with the selected yeast in the shortest time possible, in order to evade the action of bacteria. The conditions dealt with involved the study of the different hydrogen ion concentrations in the yeast media, the amount of yeast necessary for the complete exhaustion of a given amount of a fermentable sugar, the different sugars the selected yeast can digest, the time required for the completion of the fermentation process, and some other technical points.

The following technique was finally adopted: 10 cc. of 10% washed yeast, 2 cc. acetate buffer (pH 4.7), 2 cc. 0.2 M KH_2PO_4 and 25 cc. sugar solution of known strength are mixed in a 50-cc. measuring flask and incubated at 35° C. for three hours. At the expiration of this period the mixture is neutralized to phenol red with N/10 NaOH, 4 cc. alumina cream are added with subsequent shaking of the flask, the volume is then completed up to 50 cc., and the contents filtered dry. A few drops of toluene are added to the clear filtrate which is set aside for estimation.

Very much smaller concentrations of yeast were found adequate for the complete removal of glucose, fructose, and sucrose in the time stated; maltose fermentation, on the other hand, was much slower. Using the standard concentration it was found that of 50 mg. maltose supplied, 90% had been removed in three hours; while practically complete removal in the same period was achieved when 5 cc. of 5% Taka-diastase solution were added to the yeast suspension.

Trials with galactose and the pentose sugars (xylose and arabinose) under the standard conditions showed that of 35 mg. supplied 96%, 98%,

[1] The variety used in this work is known in England by "Early Market."

and 95% of the original amounts of these sugars were recovered, respectively. The small losses are presumably due to the development of bacteria under the conditions of the incubation, since estimations after twenty-four hours incubation gave very much higher losses; a result which makes HEINZE and MURNEEK's (5) practice of 48 hours incubation and that of RYGG (11) of 18 hours incubation for fractionating yeast fermentable and non-fermentable parts of plant extracts rather questionable.

Any loss of reducing power due to the absorption, by yeast, of some of the sugars under investigation can be neglected, since experiments in which the fresh yeast was precipitated with alumina cream, immediately after mixing with the sugar solutions, gave satisfactory percentage recovery: maltose, 99%; galactose, 100%; arabinose, 100.5%; and xylose, 99%. Glucose, however, under the same treatment gave a recovery of only 96%, a result which is ascribed to its rapid fermentation, for with boiled yeast treated in the same way the recovery was 101%.

Thus the technique adopted ensures the removal of glucose, fructose, and sucrose, leaving galactose and the pentose sugars practically untouched.

APPLICATION OF THE TECHNIQUE ADOPTED FOR THE REMOVAL OF THE FERMENTABLE SUGARS FROM CARROT TISSUE EXTRACT

Time curves for complete removal of fermentable sugars in carrot tissue extract were studied on many occasions. Because of the presence of some non-fermentable reducing substances which are continually attacked by bacteria present in the yeast suspension, no constant value for these substances could be attained at any point on the time curve. For this reason, the method which was finally adopted to ensure the complete digestion of the reducing fermentable sugars in the tissue extract was as follows.

A sample of the extract (about 20 cc.) was treated with yeast under the standard conditions, and to another aliquot sample a known amount of glucose was added; in both cases the reducing value of the clear digest was the same. This implies: firstly, that fermentable sugars in the extract are totally removed; and secondly, that nothing is present in the extract to hamper in any measurable way the action of yeast.

To rule out any possible error which might arise from the application of yeast, a blank fermentation containing all of the reagents with distilled water in place of the experimental extract, was always carried out. It is interesting to note that the titration value of this yeast blank and that of pure distilled water, were always practically the same, especially when the copper method was being employed for the estimation of reducing values.

The amount of yeast used was decreased or increased according to the reducing value of the material to be fermented, and a corresponding increase or decrease was made in the amount of alumina cream used for precipitation.

EXTRACTION AND CLARIFICATION

For the extraction of carbohydrates from carrot root tissue, the 80%-alcohol technique was employed. Extraction was carried out in a simplified

soxhlet-like apparatus until no color appeared in the drippings. This was considered the end point of extraction, according to previous work done in this laboratory. The simpler carbohydrates, such as the hexoses and the disaccharides, were sought in the alcohol extract, the more complex forms in the alcohol insoluble residue. From the extract, the alcohol was removed by distillation under reduced pressure at 50°–55° C. to avoid the possibility of any sucrose inversion, especially towards the end of distillation when the bulk was reduced to a few cc. of a syrupy fluid. This latter was then taken up in water by washing it several times, with a little warm water and clean sand each time. The washings thus collected were finally clarified.

Among the many clarifying agents used by earlier investigators (**1**), basic lead acetate was most extensively applied, followed by one of a number of such reagents as carbonates, sulphates, oxalates of sodium or potassium to remove the excess lead. Loss of reducing sugars, especially of fructose present in the extract, was noticed under such treatment by many workers, and was attributed to a destructive action of the lead salt on fructose (**7**). ENGLIS and TSANG (**2**) had experimentally shown that this loss in reducing sugars from tissue extracts, clarified with basic lead acetate, was due to the nature of the deleading agents used, and not to the lead salt. In view of the facts hitherto mentioned, many trials on clarifying carrot tissue extract were carried out, using some of the reagents most commonly used for this purpose. Two precipitants, namely basic and neutral lead acetate, were chosen. Each one of the two reagents was tried on two aliquot samples of the same extract.

TABLE I

REDUCING POWER OF THE CLEAR PLANT EXTRACT EXPRESSED AS GRAMS "GLUCOSE" PER 100 GRAMS FRESH WEIGHT OF CARROT TISSUE

CLARIFYING REAGENTS EMPLOYED	TOTAL REDUCING VALUE OF THE EXTRACT	R.V. OF THE EXTRACT AFTER FERMENTATION	R.V. OF THE FERMENTABLE PORTION OF THE EXTRACT
THE FERRICYANIDE METHOD			
(1) Basic lead acetate + disodium hydrogen phosphate	3.468	0.387	3.081
(2) Basic lead acetate + potassium oxalate	3.287	0.387	2.900
(3) Neutral lead acetate + disodium hydrogen phosphate	3.761	0.376	3.385
(4) Neutral lead acetate + potassium oxalate	3.525	0.382	3.143
THE COPPER METHOD			
(1) Basic lead acetate + disodium hydrogen phosphate	3.294	0.340	2.954
(2) Basic lead acetate + potassium oxalate	3.220	0.336	2.884
(3) Neutral lead acetate + disodium hydrogen phosphate	3.519	0.323	3.196
(4) Neutral lead acetate + potassium oxalate	3.229	0.312	2.917

The excess of lead was removed from each member of the two pairs of aliquots with one or the other of the two deleading agents chosen; namely, disodium hydrogen phosphate and potassium oxalate. The reducing power of the aliquots was then estimated before and after fermenting the clarified extract with yeast. The estimation was carried out with both the ferricyanide (**3**) and the copper (**12**) methods, elaborated and modified in this laboratory for measuring the reducing power of plant extracts.

The results presented in table I show that the application of lead acetate, either basic or neutral, followed by disodium hydrogen phosphate consistently resulted in higher reducing values than when potassium oxalate was used in place of phosphate; especially for the fermentable fraction of the extract which includes all the reducing sugars. These results are in accord with the conclusion of ENGLIS and TSANG (**2**).

Accordingly the treatment with basic lead acetate as a precipitant, followed by disodium hydrogen phosphate as a deleading agent, was adopted for the work on carrot tissue extracts.

ESTIMATION OF INDIVIDUAL CARBOHYDRATES IN CARROT TISSUE EXTRACT AND THE METHODS EMPLOYED

Two methods, previously modified and elaborated in this laboratory, for the estimation of reducing substances in plant extract, were used.

THE FERRICYANIDE METHOD.—This is a modification of HAGEDORN and JENSEN'S (**3**) method which was first introduced and modified in this laboratory by Hanes, and finally standardized by HULME and NARAIN (**6**) for the estimation of pure reducing sugars in solution.

THE COPPER METHOD.—This is a modification of SHAFFER and HARTMAN'S (**12**) method which was later standardized by MASKELL and NARAIN (work hitherto unpublished). The principal modification consists in the use of a less alkaline solution and the removal of oxygen from the mixture of extract and copper reagent before boiling, by means of a stream of nitrogen gas. Under these conditions there is no re-oxidation of reduced copper during boiling, and the thiosulphate titration value is proportional to the amount of sugar present, up to a maximum of 1.5 mg. of the reducing substance (hexose) to be estimated.

Each of the two methods proved to have some characteristic merits. The ferricyanide has an advantage over the copper method in the fact that it is not affected by any oxygen that may be present in the experimental solution. Also larger amounts of reducing substances can be estimated by the ferricyanide than by the copper method; the upper limits being 3.0 and 1.5 mg., respectively. On the other hand, the copper method, relative to monosaccharides, is less sensitive to reducing disaccharides than is the ferricyanide method. Consequently, with the copper method, the rise in the reducing value after the hydrolysis of disaccharides, will be relatively greater and hence more accurately estimated, than with the ferricyanide method.

In view of the differences between the two methods and the advantages

each one has over the other in some respects, both were simultaneously used during the early part of this work. When the identity of the individual members of the carbohydrate group present in carrot tissue became well established, the copper method was chosen, being more suited to carry on with the rest of the estimations for this work (**9**). However, the ferricyanide method was called on whenever the necessity arose, to check results obtained by the copper method.

Estimation of individual carbohydrates in carrot tissue extract

The aldoses and ketoses or apparent hexoses.—Among the methods used for the estimation of aldose sugars is the iodometric one, elaborated by Romijn (**10**) and based on the oxidation of aldoses by iodine in alkaline solution. Maskell and Narain (unpublished data) found this method to give correct results for mixtures of pure sugars; for leaf extracts, however, the figures for aldose sugars, indicated by the iodine used up, were invariably much too high.

In the present work, the method was tested on carrot tissue extract. The residual iodine in the experimental samples was titrated with N/10 sodium sulphite and the difference between the values thus obtained and a blank estimation, gave values in terms of Na_2SO_3 equivalent to the I_2 reduced by the experimental solutions. Table II contains a few sets of results which represent values for the aldoses estimated by the iodometric, ferricyanide, and the copper methods. In using the two latter methods for the estimation of the aldoses, samples of the extract were treated with iodine as mentioned above, merely to remove the aldoses; then the ketoses, presumably left untouched, were estimated. The difference between the reducing value of the extract both before and after the iodine treatment gave the value for the aldoses. The extract used for this investigation was previously hydrolyzed with N/2 HCl for increasing lengths of time at 60° C. during the course of elaborating techniques for sucrose estimation. The figures obtained and presented in table II are in accord with Narain's (**8**) results working on pure sugars and ivy leaf extracts.

In view of the excessively high estimates obtained for apparent glucose (aldoses) with the iodometric method, the use of this method for accurate estimation of these sugars was ruled out. It not only falsifies the figures for aldoses, but it also leads to fictitious results for ketoses which are to be obtained by subtracting the aldose value from the total reducing value of the two, estimated jointly by either the ferricyanide or the copper method. Despite the limitations of the iodometric method and in view of the fact that ketoses are hardly oxidized by iodine under these conditions, the iodometric method was made use of merely as a means of getting the aldoses present in the extract inactive by oxidation, leaving the residual ketoses to be estimated by either of the standard methods. The coupling of the iodometric method with the standard reduction methods, proved very valuable in obtaining

reliable and accurate results. Trials for recovering pure sugars (glucose, fructose, and sucrose) added to the tissue extract proved satisfactory.

THE TRUE HEXOSES—GLUCOSE AND FRUCTOSE.—Some reducing substances that may be mistaken for glucose and fructose proved to be always present in carrot tissue extract; since these substances proved to be completely resistant to yeast fermentation, the yeast fermentation technique was applied, as described previously, to fractionate the reducing substances in the extract into non-fermentable and fermentable fractions. This latter was taken to represent the joint value of true glucose and true fructose. Practically in every case where the yeast fermentation technique was applied, and before applying the estimates of the non-fermentable substance to the interpretation of experimental results, a test was carried out to ensure the complete removal of the fermentable sugars, without prolonging the standard fermentation

TABLE II

THE EXPERIMENTAL MATERIAL WAS HYDROLYZED WITH N/2 HCl AT 60° C. FOR VARIED LENGTHS OF TIME BEFORE OXIDATION WITH IODINE

MATERIAL USED	GRAMS APPARENT GLUCOSE PER 100 GRAMS F. WT. ESTIMATED BY THE:		
	IODOMETRIC	FERRICYANIDE	COPPER
20-cc. extract hydrolyzed for 4 minutes	3.735	2.617	2.720
8 "	.....	2.123	2.991
16 "	2.820	2.655	2.220
30 "	3.100	2.800	2.632
60 "	4.230	2.515	2.861
Results of another set from a duplicate extract			
20-cc. extract hydrolyzed for 4 minutes	5.072	2.158	3.365
8 "	4.795	2.656	3.487
16 "	5.640	3.578	3.139
30 "	5.215	3.559	3.347
60 "	4.650	3.325	3.295

period. This involved the fermentation of an aliquot sample of the extract to which a known amount of pure glucose or pure fructose was added. In almost all cases, the results obtained after fermenting the entire and mixed extracts, agreed fairly well. In cases when such an agreement was not attained, however, the quantity of yeast employed was increased to effect such agreement.

Although the joint value for true glucose and true fructose present in carrot tissue extract could be obtained according to the technique so far described, no accurate separate values for the two hexoses could possibly have been obtained without firstly ascertaining whether this non-fermentable fraction belonged to the aldo or to the keto type of reducing substances, or whether it was a mixture of the two; and secondly, estimating the value of each component of a mixture.

ALDO AND KETO NON-FERMENTABLE SUBSTANCES.—To separate the non-fermentable substances into aldo and keto fractions, 20- to 30-cc. samples of the

extract were fermented under the conditions of the standard method, and the reducing value of the fermented extract was estimated. Samples of the fermented extract were then oxidized with iodine and the reducing value of the oxidized solution was again measured. This latter value was taken to represent the keto non-fermentable substance, and the difference between this and the total value (before oxidation but after fermentation) to represent the value for the aldo non-fermentable substance. By subtracting these two figures from those representing the total ketoses and total aldoses (apparent hexoses, respectively) the values for true fructose and true glucose were obtained. This procedure with a slight modification, was duplicated for confirmation. The same steps were performed, only with their sequence reversed; namely, oxidation preceding fermentation. With both sequences fairly good agreements were obtained. The results obtained at this stage and throughout the present work, indicated that the non-fermentable matter present in carrot root extract is composed of two different types; i.e., a keto and an aldo, and that the keto type constituted the major part; at times the aldo type was entirely absent. Nothing like a constant ratio between the relative values of these two substances did exist at any time. This realization, coupled with the fact that the value of the non-fermentable fraction in the extract was by no means too low to be ignored, rising at times to more than 10% of the value for true hexoses, necessitated going through the tedious processes of their fractionation whenever the estimation of true sugars was required.

Before passing to the estimation of other members of the carbohydrate group present in carrot tissue extract, a few trials were carried out to check the reliability of the figures so obtained in representing the true hexoses in that extract. Recovery of pure fructose added to the extract was tried, both before and after treating the mixture with iodine. Within the limits of experimental error, 100% recovery was obtained, suggesting that nothing in the carrot tissue extract, nor the treatment with iodine, affects the recovery of pure sugar added to the extract in any measurable degree, and hence the genuineness of the figures for glucose and fructose present in this extract may be accepted.

THE DISACCHARIDES—SUCROSE.—The method adopted in this laboratory and applied to pure sucrose and leaf extracts was based on hydrolyzing the material to be analyzed for sucrose, with half normal hydrochloric acid at 60° C., and measuring the reducing value after hydrolysis.

Since the value for sucrose in carrot tissue extract was to be obtained from the increase in the reducing value of the extract after hydrolysis with N/2 HCl at 60° C., the accuracy of such a value would necessarily depend on, firstly, whether the non-fermentable reducing substances in the extract would undergo any substantial change under the conditions of hydrolysis; and, secondly, whether any disaccharide, other than sucrose, may be present in the extract to augment the reducing value of that extract on hydrolysis.

A few trials were carried out to test the behavior of the non-fermentable

TABLE III

Material	Reducing values as mgs. glucose in 20 cc. tissue extract estimated by:			
	The ferricynide method		The copper method	
	mg.		*mg.*	
20-cc. extract fermented	15.80	.....	13.6	.....
20-cc. extract hydrolyzed and fermented	10.00	37*	9.4	31*

* Percentage loss.

reducing substances under these conditions. Samples of the extract were fermented and other aliquot samples were hydrolyzed and then fermented. Results obtained and recorded in table III clearly show a consistent loss of the non-fermentable material; a loss which suggests that the material in question had either undergone partial destruction under the prevailing conditions, or had been partially hydrolyzed into some substance fermentable by the yeast used. To clear up this point, samples of the extract were fermented, and a part of the fermented extract hydrolyzed with N/2 HCl at 60° C. The result thus obtained and recorded in table IV indicates that the non-fermentable reducing matter in carrot tissue extract undergoes partial destruction under the conditions of hydrolysis with N/2 HCl. Although the loss in the non-fermentable substance amounted to over 30% at times, the total value of this substance relative to the total reducing power of the carrot tissue extract (after hydrolysis) is quite small, the allowance for this destruction can be safely ignored without appreciably affecting the results for sucrose.

To find out whether sucrose was present and if so, whether it was the only disaccharide that occurs in carrot tissue, a study of the hydrolysis time curve was carried out. Such a curve would also indicate the period necessary for hydrolysis to attain completion. For this purpose two comparable tissue samples A and B were separately extracted and clarified. From the clear

TABLE IV

Material	Reducing values as mgs. glucose in 20 cc. tissue extract estimated by:			
	The ferricyanide method		The copper method	
	mg.		*mg.*	
20-cc. extract fermented	15.2	.....	12.6	.....
20-cc. extract hydrolyzed and fermented	12.2	20*	10.0	20*

* Percentage loss.

extracts, two sets of samples, one set for each extract, were hydrolyzed with N/2 HCl at 60° C. for varied lengths of time, and their reducing values estimated both before and after hydrolysis with both the two standard methods. The results obtained are presented in table V. It is evident from these results, that the total reducing value of the extract rose rapidly with time, until a level was reached after 16 minutes, beyond which time no further increase or decrease took place.

TABLE V

ANALYSIS OF TWO DUPLICATE EXTRACTS A AND B
RESULTS ARE EXPRESSED AS GRAMS APPARENT HEXOSES PER 100 GRAMS FRESH WEIGHT

METHOD OF ESTIMATION	MATERIAL USED		TOTAL R.V. AS APPARENT GLUCOSE (1)	APPARENT FRUCTOSE (2)	APPARENT GLUCOSE (3)
			gm.	*gm.*	*gm.*
The copper	Unhydrolyzed extract	A.	3.780	1.710	2.070
		B.	3.785 } 3.783	1.840 } 1.775	1.945 } 2.008
	Hydrolyzed at 60° C. for:				
	4 minutes	A.	5.375	2.788	2.587
		B.	6.020 } 5.698	2.725 } 2.759	3.295 } 2.941
	8 "	A.	6.160	3.022	3.138
		B.	5.960 } 6.060	3.210 } 3.116	2.750 } 2.944
	16 "	A.	6.355	3.140	3.215
		B.	6.320 } 6.338	3.007 } 3.074	3.313 } 3.264
	30 "	A.	6.250	3.125	3.125
		B.	6.425 } 6.338	3.195 } 3.160	3.230 } 3.178
	60 "	A.	6.310	2.808	3.502
		B.	6.420 } 6.365	3.150 } 2.979	3.270 } 3.386
The ferricyanide	Unhydrolyzed extract	A.	3.995	2.180	1.815
		B.	4.332 } 4.164	2.343 } 2.262	1.989 } 1.902
	Hydrolyzed at 60° C. for:				
	4 minutes	A.	5.880	3.338	2.542
		B.	6.960 } 6.420	3.050 } 3.194	3.910 } 3.226
	8 "	A.	6.930	3.562	3.368
		B.	7.032 } 6.981	3.550 } 3.556	3.482 } 3.425
	16 "	A.	7.465	3.575	3.890
		B.	7.365 } 7.415	3.435 } 3.505	3.930 } 3.910
	30 "	A.	7.135	3.587	3.548
		B.	7.440 } 7.288	3.607 } 3.599	3.833 } 3.691
	60 "	A.	7.185	3.475	3.710
		B.	7.570 } 7.378	3.300 } 3.388	4.270 } 3.990

To identify and fractionate the hydrolysis products, the reducing values of the extract, before and after hydrolysis, were resolved into ketose and aldose fractions, and the results obtained were again recorded in table V under columns 2 and 3, respectively.

Since the maximum reducing value of the hydrolysis products was attained after 16 minutes and remained constant beyond that time, the values obtained for each set (A and B) in 16, 30, and 60 minutes were treated as comparable estimates of the hydrolysis products, and accordingly their mean

for each set, as well as for the two sets combined, was taken to give a more accurate estimate for 100% hydrolysis. In table VI, column 2, the means of the three last estimates for the hydrolysis products, differentiated into aldoses and ketoses are presented. In the same table, column 1, are also tabulated the aldoses and ketoses initially present in the extract. Since no allowance for destruction in the non-fermentable matter was involved at this stage, the difference between the corresponding values in columns 1 and 2 was taken to represent true glucose and true fructose that resulted from the hydrolysis of the substance or substances under investigation. These values for true glucose and fructose are shown in column 3.

The results obtained by the copper method (table VI) suggest that the products of hydrolysis, within the limits of experimental error, are composed of equal quantities of true glucose and true fructose. By the ferricyanide

TABLE VI

REDUCING VALUES AS GRAMS HEXOSE PER 100 GRAMS FRESH WEIGHT

METHOD OF ESTIMATION	MATERIAL	APPARENT HEXOSE				INCREASE IN TRUE HEXOSES DUE TO HYDROLYSIS	
		INITIAL		AFTER HYDROLYSIS			
		(1)		(2)		(3)	
		KETOSES	ALDOSES	KETOSES	ALDOSES	FRUCTOSE	GLUCOSE
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Copper	Set A	1.71	2.07	3.02	3.28	1.31	1.21
	Set B	1.84	1.95	3.12	3.27	1.28	1.32
	Mean (A & B)	1.775	2.01	3.07	3.275	1.295	1.265
Ferricyanide	Set A	2.18	1.82	3.55	3.72	1.37	1.90
	Set B	2.34	1.99	3.45	4.01	1.11	2.02
	Mean (A & B)	2.26	1.905	3.50	3.865	1.24	1.96

method, however, glucose is consistently higher than fructose; a discrepancy which is quite inexplicable and must be ascribed to some failing in that method rather than to a substance in the extract responsible for this excess of glucose. This assumption is substantiated by the fact, as mentioned earlier, that the copper method relative to hexose is less sensitive to disaccharides than is the ferricyanide method; hence it follows that in case of the presence of a substance of this category in the extract, the copper method should give—after hydrolysis—higher reducing values relative to the initial reducing power of the extract, than would the ferricyanide method. In no instance was this the case; on the contrary, the ferricyanide method invariably gave higher results. Nevertheless, an attempt was made to find a cause for this disparity between the two components (glucose and fructose) of the hydrolysis products, as estimated by the ferricyanide method; a cause which was thought to lie probably in the oxidation reagents and their possible interference with the estimation of ketoses—and consequently the aldoses. After many fruitless efforts, through which it was well established that the oxida-

tion reagents did in no way interfere with the estimation of the reducing power of carrot extracts no such cause was discovered to account for the behavior of the ferricyanide method in this respect.

Since the rate of hydrolysis of any material under any given set of conditions is a fairly good index to its identification, the rate of hydrolysis of carrot root extract under these conditions was calculated, in the hope of shedding some light on the nature of the substance in question. The results of calculation are shown in table VII. The mean value of the last three esti-

TABLE VII

PERCENTAGE AND RATE OF HYDROLYSIS

TIME OF HYDROLYSIS	METHOD OF ESTIMATION					
	THE COPPER METHOD			THE FERRICYANIDE		
	INCREASE IN R.V. GLU. + FR.	HYDROLYSIS		INRCEASE IN R.V. GLU. + FR.	HYDROLYSIS	
		PERCENTAGE	RATE*		PERCENTAGE	RATE
		%			%	
4 minutes	1.915	74.7	0.37	2.257	70.6	0.31
8 "	2.278	88.9	"per	2.818	88.2	"per
16 "	2.555	99.7	minute"	3.252	101.7	minute"
30 "	2.555	99.7		3.124	97.8	
60 "	2.583	100.7		3.214	100.6	
Mean of 16, 30, and 60 minutes	2.564	100.0		3.197	100.0	

* Rate of hydrolysis was worked out according to the equation (4)

$$K = \frac{1}{t} \log \frac{a}{a-x}$$

Where K is rate of hydrolysis in t time: a the amount of cane-sugar present at the start, and x the amount of cane-sugar that is hydrolyzed.

mates for the hydrolysis products (after 16, 30, and 60 minutes) was taken to represent 100%. Accordingly, percentage hydrolysis after varied lengths of time, and also the rate of hydrolysis [after HARVEY (4)] were calculated.

It is evident that the results obtained for the products of hydrolysis at 60° C. with N/2 HCl, show that:

1. The hydrolysis time curve attained a maximum value after 16 minutes, beyond which time it kept practically level.

2. The hydrolysis products are composed entirely of glucose and fructose, as measured by the copper method.

3. The rate of hydrolysis of the extract is of the same order as that for pure sucrose under the same conditions.

Such conclusions strongly suggest that sucrose is the only disaccharide present in carrot tissue extract. Accordingly, the technique adopted for the estimation of sucrose in this work, was based on treating the extract with N/2 HCl at 60° C. for 30 minutes.

MALTOSE.—The method which has been used in this laboratory by MASKELL and NARAIN (unpublished data) for the estimation of maltose, based on the hydrolysis of this sugar with N HCl at 100° C. and allowing for the destruction of the hydrolysis products at the rate of 1% per hour, was tried on carrot tissue extract; also hydrolyzing the extract with 0.5% Taka-diastase was attempted. Results obtained are recorded in table VIII, together with the reducing value of the same extract after N/2 HCl hydrolysis at 60° C. The increase due to hydrolysis after both the treatment with N/2 HCl at 60° C. and with 0.5% Taka-diastase is practically the same. The low results obtained after hydrolysis with N HCl at 100° C. might be due to a greater rate of destruction than has been allowed for. These results further confirm the conclusion, already arrived at; namely, that sucrose is the only disaccharide present in carrot root tissue.

TABLE VIII

REDUCING VALUES OF CARROT TISSUE EXTRACT AS GRAMS TRUE HEXOSES PER 100 GRAMS FRESH WEIGHT BEFORE AND AFTER DIFFERENT TREATMENTS*

REDUCING VALUES	HYDROLYZING AGENT:		
	N/2 HCl AT 60° C.	N HCl AT 100° C.	0.5% TAKA-DIASTASE AT 35° C.
	gm.	*gm.*	*gm.*
T.R.V. before hydrolysis	4.165	4.155	4.200
T.R.V. after "	7.365	6.906	7.403
Increase " "	3.200	2.751	3.203

* Figures shown in this table are means of values obtained for the duplicate extracts A and B by the ferricyanide method.

To conclude: here follows a short account of the steps followed for a complete analysis sheet of individual carbohydrates, present in the 80% alcohol extract of carrot tissue, according to the technique hitherto discussed:

STEPS	TREATMENT	RESULTS
A.	Extract directly estimated	Direct total reducing value
B.	Extract fermented with yeast and estimated	Total non-fermentable reducing matter
C.	Fermented extract oxidized with iodine	Keto non-fermentable reducing matter
D.	Extract oxidized with iodine	Total direct reducing ketoses
E.	Extract hydrolyzed with N/2 HCl at 60° C.	Total reducing value after hydrolysis
F.	Hydrolyzed extract oxidized with iodine	Total reducing ketoses after hydrolysis

Thus

A–B = Total fermentable reducing matter, or true hexoses
B–C = Aldo non-fermentable reducing matter
A–D = Total direct reducing aldoses

A–D–(B–C) = Fermentable direct reducing aldoses or true glucose
D–C = Fermentable direct reducing ketoses or true fructose
E–A = Total increase in fermentable reducing matter or the increase in glucose and fructose due to inversion
E–F = Total reducing aldoses after inversion
F–D = Increase in fructose due to inversion
E–F–(A–D) = Increase in glucose due to inversion

All the results obtained according to the above scheme represent reducing values in terms of glucose, and accordingly (D–C) and (F–D) in order to represent fructose, must be corrected by adding 3%; fructose being that amount lower in its reducing power than glucose, as estimated by the copper method. Hence it follows:

$$\frac{(D-C)\times 100}{97} = \text{Initial fructose}$$

$$\frac{(F-D)\times 100}{97} = \text{Increase in fructose after inversion}$$

$$\frac{(F-D)\times 100}{97} + E-F-(A-D) = \text{Sucrose as invert sugar}$$

$$\frac{342}{360}\times\left(\frac{(F-D)\times 100}{97} + E-F-(A-D)\right) = \text{Sucrose as such}$$

Summary

1. Carrot tissue was extracted with 80% boiling alcohol. The aqueous extract was cleared with basic lead acetate and disodium phosphate.

2. Using the two reduction methods (the copper and ferricyanide) glucose, fructose, and sucrose were found and estimated in the clear extract. Maltose was entirely absent.

3. Trials for fermenting various sugars with ordinary baker's yeast were attempted. Glucose, fructose, and sucrose were completely fermented in less than three hours. Maltose was more resistant than the three mentioned sugars. It was almost equally rapidly fermented when a solution of Taka-diastase was added to the yeast suspension. The pentoses, arabinose and xylose, were not attacked by the yeast used.

4. The yeast technique was applied to carrot tissue extract for separating the non-fermentable reducing matter which was generally taken for reducing sugars.

This work was carried out at the Botany School, Cambridge, England. The author wishes to express his indebtedness to the late Dr. F. F. Blackman, F.R.S., for his kind encouragement and to Dr. E. J. Maskell, F.R.S., for his intense interest in the work and his continual advice and criticism. The author further wishes to thank Mr. C. Sewell for his help with the analyses.

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RESPIRATION AND NITROGEN METABOLISM OF WHOLE AND SLICED RADISH ROOTS WITH REFERENCE TO THE EFFECT OF ALTERNATION OF AIR AND NITROGEN ATMOSPHERES[1]

HUSEIN SAÏD AND E. D. H. EL SHISHINY

(WITH FOUR FIGURES)

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Introduction

It has long been known that wounded plants respire more intensely than intact ones (**3, 5, 6, 13, 14, 17, 18, 22, 23, 29, 30, 31, 32**).

AUDUS (**1, 2**), and GODWIN (**10**) reported that handling of plant parts, without apparent wounding, causes great increase in their rate of respiration. The stimulus that causes this rise in respiration arises apparently from deformation of cells by bending or rubbing. MILLER (**19**) states that the marked increase in the output of carbon dioxide following injury is probably due to the escape of the gas that has accumulated in the intercellular spaces of the tissues. There is no doubt that injury of bulky organs renders the atmospheric oxygen more accessible across the tissues (**3, 18, 19, 27, 32**).

TURNER (**31**) observed that when carrot roots are sliced into 1-mm.-thick discs, the latter usually give a high initial rate of respiration which exhibits wide variations but with general tendency to fall. After 50 hours, the fall in respiration becomes more regular until after 150–200 hours, a rate is reached which is about the same as that of intact carrot root. The high initial respiration of the carrot slices is ascribed to stimulation caused by cutting, washing, and handling.

BENNET-CLARK and BEXON (**3**) found that with continued washing in aerated tap water, the respiration rate of beet root slices rises from a very low value shortly after cutting to a high maximum value after some 300 hours' washing, a value which is then more or less maintained for another 350 hours. The authors suggest that the rise in the respiration of the slices and the maintenance of high rate for many days is due to less restricted gaseous exchange and to mechanical injury and wound stimuli which may induce metabolic changes leading to an increased concentration of respirable substrate or of respiration enzymes.

STILES & DENT (**30**), measuring the respiration activity of discs from various storage tissues, found that on first cutting out the slices the respiration starts low and rises slowly in aerated running tap water to a much higher level which may be maintained constant for a time, but from which it slowly diminishes. These two authors agree with BENNET-CLARK and

[1] This is the fourth of a series of papers on the general subject of Researches on Plant Metabolism.

BEXON (3) in the explanation of the gradual rise of the respiration activity as a consequence of separation of thin slices from a storage organ, and they further consider the subsequent fall in respiration rate to be due to exhaustion of the respirable substrate or to the slow development of some depressant factor.

Some of the metabolic changes that occur as a result of injury of plant organs have been studied. Thus GRUSS (12) observed that wounding of potato induced sugar accumulation and increased oxidizing enzymes and diastatic activity in the cells around the wound. FRIEDRICH (9) noted in the cells bordering the cut surfaces of potato an increase in the total nitrogen and reducing sugars. HOPKINS (15) showed that wounding caused disappearance of starch and increase in sugar content; the latter resulted in increase of respiration activity. LUTMAN (18), on the other hand, was of the opinion that the increase in sugar concentration had no relation with the increase in respiration of wounded tissues.

Recent work seems to indicate that respiration may be more closely connected with nitrogen metabolism than with carbohydrate changes (11, 16, 21, 28). ZALESKI (34) claimed that wounded storage organs always increased their protein content, presumably at the expense of their soluble nitrogen.

The aim of the present paper is to investigate the changes in the respiration activity resulting from the separation of thin slices from roots of *Raphanus sativus Aegyptiacus*, and to see if these changes would be accompanied by variations in the nitrogen fractions of the plant.

Experimentation and results

The carbon dioxide given off in respiration was absorbed in Pettenkofer tubes and then estimated titrimetrically. By the use of a battery of Pettenkofer tubes and a commutator designed by F. F. Blackman, a continuous day and night record of respiration could be obtained. The methods used for the estimation of the various nitrogen fractions have already been referred to in a previous paper (25).

Mature radish plants were usually brought to the laboratory early in the morning; the leaves were removed, the roots thoroughly washed with tap water and then wiped dry. In experiments on whole roots, the fresh weights of the roots were first determined; and then the cut ends were sealed with wax of low melting-point. Slicing of the roots was always carried out by means of a special sledge microtome. The following experiments are typical representatives of many repetitions and the results reported have been fully confirmed.

FIRST EXPERIMENT

RESPIRATION AND NITROGEN METABOLISM OF WHOLE RADISH ROOTS.—Four more or less similar roots were sterilized by means of freshly prepared solution of 6.66 per cent of calcium hypochlorite as recommended by WILSON

(33) and PEARSALL and BILLIMORIA (20), and then washed with sterilized distilled water. After that two roots were extracted with boiling distilled water and immediately analyzed for the various initial nitrogen fractions. The other two roots were placed in two respiration chambers which were then submerged to the neck in a water-bath whose temperature was kept constant at 25° C. by means of a toluene-mercury thermo-regulator. A carbon-dioxide-free air was passed through the chambers at a rate of two liters per chamber per hour. Allowance of two hours was always given for the sweeping of all the carbon dioxide present in the apparatus, and also for the plant material to adapt itself to the temperature of the bath. After that the carbon-dioxide outputs by the two roots were estimated at 8-hour intervals for 120 hours. At the end of the experimental period, the two samples were extracted in boiling water and analyzed for their nitrogen fractions.

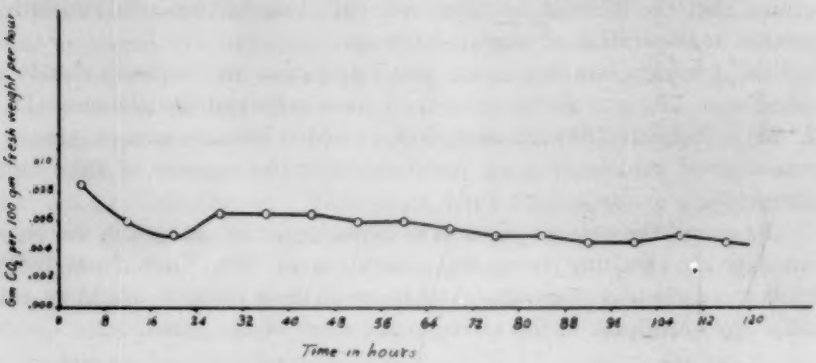

FIG. 1. Drift in the respiration rate of mature whole radish roots.

RESPIRATION OF WHOLE ROOTS.—The average carbon-dioxide output of the two samples is represented graphically in figure 1, being calculated as grams CO_2 given off by 100 grams fresh weight of roots per hour. The results of this as well as many similar experiments showed that the respiration of whole radish roots always proceeds at a low but steady rate for prolonged periods.

ANALYSIS OF TISSUES.—The results obtained from the analysis of the radish roots for their nitrogen fractions are recorded in table I. It is clear from this table that very little change, if any, takes place in the nitrogen fractions during 120 hours' starvation of mature radish roots. It is interesting to note that small but consistent inverse changes are always observed in the ammonia and amide-N fractions.

SECOND EXPERIMENT

RESPIRATION OF WHOLE AND SLICED ROOTS.—The respiration of two whole roots was determined in the usual way as in the first experiment except that the carbon-dioxide outputs were estimated at 5-hour intervals. After 45 hours, when the steady drifts in the rates of respiration of the two samples

TABLE I

CHANGES IN THE NITROGEN FRACTIONS OF WHOLE RADISH ROOTS RESPIRING IN AIR FOR 120 HOURS, ALL BEING CALCULATED IN MILLIGRAMS PER 100 GRAMS FRESH WEIGHT

SAMPLES	TIME OF ANALYSIS IN HOURS	NITRATE N	AMMONIA N	AMIDE N	OTHER N	TOTAL SOLUBLE N	PROTEIN N	TOTAL N
	hrs.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
A	0	40.5	4.5	5.8	66.6	117.4	45.2	163.6
B	0	45.2	5.1	5.9	63.5	119.7	48.7	168.4
C	120	45.5	2.7	9.2	60.8	118.2	44.0	162.2
D	120	44.9	3.6	7.4	66.1	122.0	42.5	164.5

were ascertained, one root (sample A) was sliced, and the slices were suspended in 200 ml. of sterilized distilled water through which air was passed. The carbon-dioxide outputs by the whole and sliced roots were then followed for another period of 80 hours. The drifts in the respiration rates of both whole and sliced roots throughout the experimental period of 125 hours are presented in figure 2, all being calculated as grams carbon dioxide given off per 100 grams fresh weight of tissue per hour.

During the first 45 hours the two roots showed similar low but steady respiration rates as in the previous experiment. The period immediately following the slicing of sample (A) was characterized by irregular increases in the carbon-dioxide output by the sliced root. The respiration of the other whole root continued at a low steady rate with very slight tendency to fall.

It is interesting to note that the respiration rate of the sliced root reached

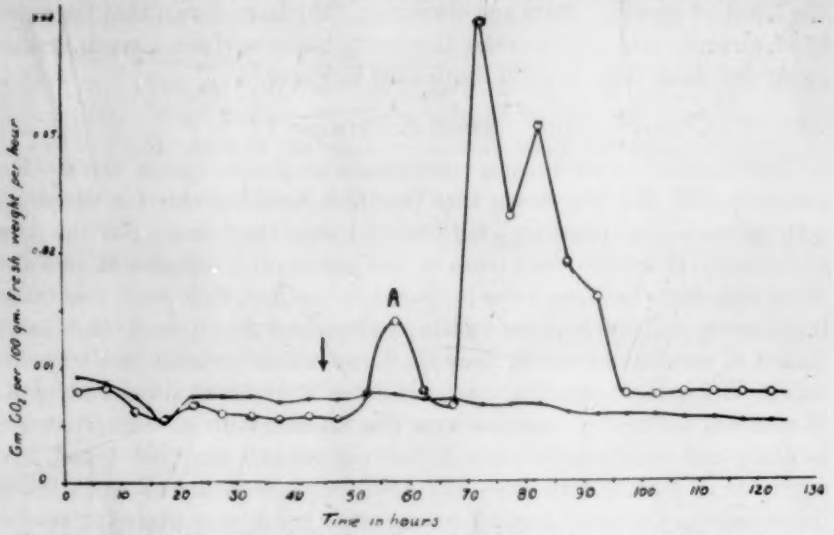

FIG. 2. Respiration rates of whole and sliced radish roots; arrow indicates the point at which slicing of sample A took place.

its maximum 30 hours after slicing and then subsided fairly rapidly for 20 more hours, after which it reached a steady level course parallel to that of the intact root, though slightly higher. The results of this and many similar experiments agree well with the findings of STILES and DENT (**30**) using slices from various storage organs. It may be recalled here that BENNET-CLARK and BEXON (**3**) failed to record the decline in the respiration intensity of the slices after reaching its maximum, and TURNER (**31**) did not observe the rise of the respiration rate of slices from a low value to a maximum.

The rise in respiration rate obtained after cutting out the discs has been attributed to more free gaseous exchange (**3, 19, 30, 32**), to mechanical and wound stimuli (**1, 2, 10, 18, 31**) which may induce internal metabolic changes leading to increased concentration of respiratory substrate or of respiratory enzymes (**3, 9, 12, 15, 30**).

The fall in respiratory activity of the slices after reaching its maximum has been considered by STILES and DENT (**30**) as due to exhaustion of respiratory substrate or to slow development of some depressant factor. The former seems to be the more probable explanation since STILES and DENT (**30**) have shown that the respiratory quotient of beet root slices and other tissues decline from 1 down to 0.85 under conditions leading to starvation; and this has been attributed to the utilization of proteins after the depletion of the carbohydrate substrate. Relevant to this point it may be stated that feeding the radish root slices with sugar causes a great increase in the respiration intensity of these slices well above that of the slices in distilled water (**25**). It must be remembered that while the more free accessibility of oxygen across the root slices tends to increase their respiration, the exhaustion of the available respirable substrate opposes and may even mask the effect of oxygen. SAÏD and SHISHINY (**25**) have shown that the increase in respiration rate with increase in specific tissue surface is much greater in sugar-fed slices than in slices suspended in water.

THIRD EXPERIMENT

RESPIRATION AND NITROGEN METABOLISM OF SLICED RADISH ROOTS.—Previous work (**24, 25**) has shown that the slices most suitable for the study of respiration and metabolism are those of 1 mm. thickness. For the present experiment, therefore, four more or less comparable samples of radish root slices of 1 mm. thickness were prepared in the following way: two consecutive discs were dried between blotting papers and then halved; each half was placed at random in one of four tin boxes whose weights had been determined, this procedure continued until about 20 grams of slices were obtained in each box. The four samples were first washed with calcium hypochlorite solution and then with sterilized distilled water. Samples 1 and 2 were extracted in boiling water and analyzed for their initial nitrogen fractions immediately. Samples 3 and 4, on the other hand, were placed in two respiration chambers, each containing 200 ml. of sterilized distilled water through which carbon-dioxide-free air was passed, and their carbon-dioxide outputs

were measured at 5-hour intervals. After 50 hours, when the respiration reached its steady rate, sample 3 was removed, extracted, and then analyzed for the various nitrogenous fractions. Sample 4 was left to continue respiring for another period of 55 hours and then analyzed for its nitrogenous fractions.

RESPIRATION OF RADISH SLICES.—The respiration of sample 4 is graphically represented in figure 3, being calculated in grams carbon dioxide per 100 grams fresh weight of slices per hour. The results of this experiment confirmed those of the previous one in showing that on first separating the thin slices from the root, their respiration starts low, and then increases to a maximum rate which may be reached after 20 to 30 hours, depending upon the previous history of the plant organ and experimental conditions such as

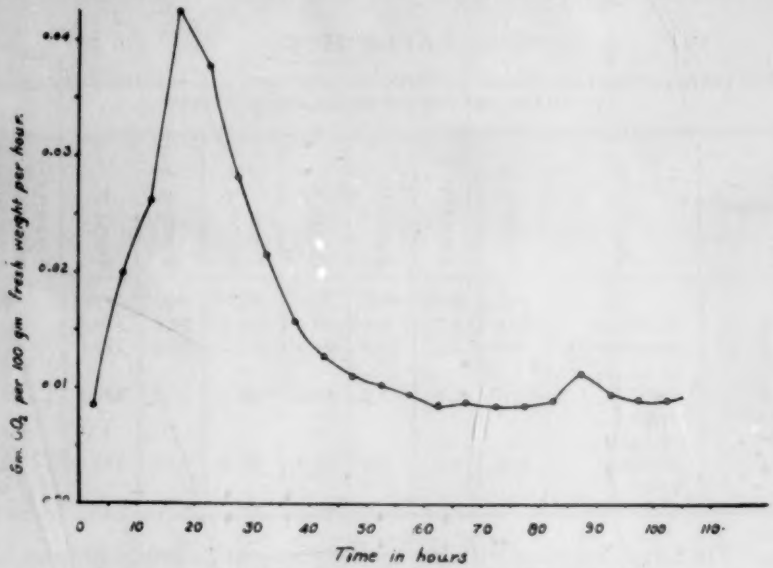

FIG. 3. Drift in the respiration rate of radish root slices.

temperature et cetera. This is followed by a subsequent rapid decline to a low steady rate which may be maintained for prolonged periods with very slight tendency to fall.

ANALYSIS OF TISSUES.—The results obtained from the analysis of the tissues for their nitrogenous fractions are given in table II. It is clear that the radish slices showed great changes in their nitrogenous fractions in the first 50 hours that followed cutting out the slices. After this period, very few changes took place in the nitrogenous fractions during the next 55 hours. It should be recalled that during this latter period the respiration of the slices was maintained at a low and steady level.

The essential change in the nitrogen metabolism that resulted from cutting out the discs was a decrease in the total soluble-N and an increase in the protein-N. The increase in the protein-N was too small to account for

the decrease in the total soluble nitrogen. Examining the mean value of the total nitrogen of the initial samples 1 and 2 and that of sample 3, it was found that sample 3 had lost 40.0 mg. nitrogen per 100 gm. fresh weight of tissues, which must have diffused out into the water medium. When the latter was analyzed, 39.3 mg. nitrogen could be recovered from the medium of 100 gm. fresh weight of slices.

It is interesting to note that although the radish slices lost a good deal of their nitrate nitrogen during the first 50 hours, yet no trace of nitrates, and insignificantly small amounts of ammonia-N, could be detected in the nitrogen recovered from the water medium. It seems, therefore, that the nitrates which disappeared from the tissues during the wounding metabolism must have been reduced and then transformed into some organic soluble

TABLE II

THE EFFECT OF ROOT SLICING ON NITROGEN DISTRIBUTION; ALL FRACTIONS ARE GIVEN IN MILLIGRAMS PER 100 GRAMS FRESH WEIGHT

SAMPLES	PROCEDURE	NITRATE N	AMMONIA N	AMIDE N	OTHER N	TOTAL SOLUBLE N	PROTEIN N	TOTAL N	NITROGEN RECOVERED FROM THE WATER MEDIA
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	Analyzed	39.5	3.7	9.7	66.4	119.3	59.6	178.9	.....
2	immediately	38.2	3.7	10.2	64.9	117.0	62.6	179.6	.....
3	50 hr. in distilled water	15.7	1.8	3.2	48.4	69.1	70.2	139.3	39.3
4	105 hr. in distilled water	14.8	1.2	6.8	44.8	67.6	70.6	138.2	40.5

form. The latter, together with the "other nitrogen" already present in the slices, might have been partly synthesized into protein and partly excreted into the external medium.

Another alternative that seemed plausible was that the protoplasm of the cut cells on the surfaces of the slices might have been disorganized and its proteins broken down into organic soluble compounds which appeared in the water medium. In the meantime, some nitrates, together with some of the "other nitrogen," were synthesized into proteins, thus compensating for all the protein of the disorganized cut cells and even exceeding the initial protein nitrogen content by 9 mg. per 100 gm. fresh weight of slices. Both alternatives might take place.

The amide nitrogen that disappeared from the slices during the first 50 hours of the experiment was most probably excreted into the medium. The increase in this nitrogen fraction during the later part of the experiment might have come from amidation of some amino-acids.

FOURTH EXPERIMENT

RESPIRATION AND NITROGEN METABOLISM OF RADISH SLICES IN ALTERNATING AIR AND NITROGEN ATMOSPHERES.—Eight more or less comparable samples, 20 grams each, of radish root slices of 1 mm. thickness were prepared. Two samples were extracted in boiling distilled water and then analyzed immediately for their initial nitrogen fractions. The remaining samples were washed in running tap-water for a few minutes and then placed in six respiration chambers, each containing 200 ml. of sterilized distilled water, and were all kept at a constant temperature of 25° C. Carbon-dioxide-free air was passed through the water in all the chambers at a steady rate of 2 liters per chamber per hour. The carbon dioxide given off by two representative samples was collected in two sets of Pettenkofer tubes and was

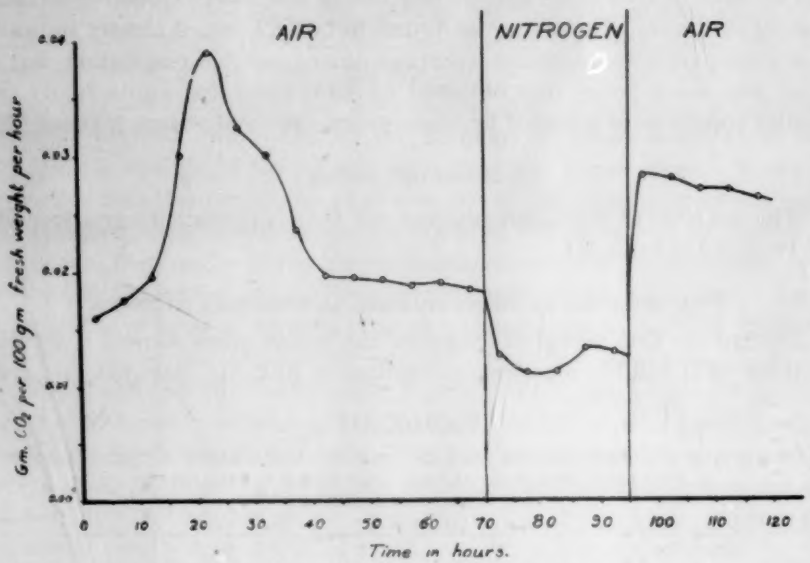

FIG. 4. Respiration rates of radish slices in alternating air and nitrogen atmospheres.

determined at 5-hour intervals. After 70 hours, two samples were drained, extracted and then analyzed for their nitrogen fractions. At the same time, pure nitrogen was passed through the remaining chambers, and the respiration rate was followed under the new conditions for a period of 25 hours. After that two more samples were extracted and analyzed while the last couple of samples were allowed to respire in carbon-dioxide-free air again for a further period of 25 hours, after which they were analyzed for their nitrogen fractions.

RESPIRATION OF THE SLICES.—The average drift in the respiration rate of the radish slices in alternating air and nitrogen atmospheres is illustrated in figure 4. It is clear that the slices first showed the respiration rate characteristic of wound-metabolism, which lasted about 45 hours, after

which the respiration rate declined to a low steady rate. At the seventieth hour, the radish slices were transferred from air to nitrogen, and their respiration rate dropped rapidly to another level below that of the air-line respiration. This lower carbon-dioxide output was more or less maintained during the period of anaerobiosis, and after 25 hours the slices were transferred back to air and their respiration rose very sharply above the air-line rate. After that the respiration continued at a steady decreasing rate. Other similar but prolonged experiments showed that the air-line respiration rate was usually reached about 48 hours after transference from nitrogen to air. It might be interesting to point out here that when the line of anaerobic respiration (fig. 4) was extrapolated back to the point of transference from air to nitrogen, the initial anaerobic respiration (INR) could be evaluated, and the ratio of INR to the aerobic respiration just before transference could thus be obtained. This ratio was found to be 0.62, which clearly indicated that some oxidative anabolism occurred during aerobic respiration, but its value was much below that obtained by BLACKMAN for apple fruits (**4**). Similar results were reported by SHOUDHURY (**26**) and STILES & DENT (**30**).

ANALYSIS OF TISSUES

The analysis of the sliced samples for their various nitrogen fractions are recorded in table III.

THE BEHAVIOR OF TOTAL SOLUBLE AND PROTEIN NITROGEN

During the first period of 70 hours, the radish slices showed a decrease in their total soluble nitrogen amounting to 38.3 mg. per 100 gm. fresh

TABLE III

THE EFFECT OF ALTERNATION OF AIR AND NITROGEN ATMOSPHERES ON THE NITROGEN FRACTIONS OF RADISH SLICES, CALCULATED IN MILLIGRAMS PER 100 GRAMS FRESH WEIGHT*

SAMPLES	PROCEDURE	NITRATE N	AMMONIA N	AMIDE N	OTHER N	TOTAL SOLUBLE N	PROTEIN N	TOTAL N	NITROGEN RECOVERED FROM THE WATER MEDIA
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1 2	Analyzed immediately	32.0	3.1	13.1	60.9	109.0	64.8	174.8	.....
3 4	70 hr. in air	17.1	1.7	5.5	46.4	70.7	78.6	149.3	30.0
5 6	70 hr. in air, then 25 hr. in nitrogen	17.2	2.4	3.8	50.0	73.4	75.9	149.3	30.3
7 8	70 hr. in air, 25 hr. in nitrogen, and then 25 hr. in air	13.7	2.4	2.5	60.1	78.7	72.9	151.6	29.6

* The values included in this table are means for duplicate samples.

weight of tissues. This decrease could be accounted for partly by some increase in the protein nitrogen and partly by the soluble organic nitrogen recovered from the external water media, thus adding further support to the results of the previous experiment.

During the following period of 25 hours, the plant tissues were respiring anaerobically, and there was a slight tendency of protein hydrolysis with corresponding increase in the soluble nitrogen content. Such protein hydrolysis, however, did not seem to be a direct result of anaerobiosis since it continued even when the nitrogen atmosphere was substituted by air in the last 25 hours of the experiment. This protein breakdown was most probably due to low carbohydrate content of the plant tissues.

The behavior of nitrate nitrogen

Similar to the findings of the previous experiment, the greatest change in the nitrate nitrogen fraction took place during the early period that followed the slicing of the roots. All the nitrates that disappeared from the tissues were not detected in the medium and were presumably synthesized into some organic form. During the 25 hours of anaerobiosis, there was no apparent change in the nitrate content of the tissue slices. It should be recalled that this period was characterized by the lowest respiration rate; and assuming that nitrate reduction involved the utilization of energy of respiration, then under anaerobic conditions the respiration must have been too low to produce the energy necessary for such nitrate reduction. Alternatively, the absence of oxygen from the atmosphere might have inhibited the action of the nitrate-reducing enzyme system [cf. Eckerson (**7, 8**)].

Transference of the radish slices from nitrogen back to air for another period of 25 hours resulted in a disappearance of 3.5 mg. of nitrate nitrogen per 100 gm. fresh weight of tissues. The high respiration obtained on transference from nitrogen to air might have afforded enough energy for the resumption of nitrate reduction, or alternatively, the reducase enzyme system might have been reactivated by the oxygen of the atmosphere.

The behavior of "other nitrogen"

During the first period of 70 hours in air, this nitrogen fraction lost 14.5 mg. per 100 gm. fresh weight of slices which probably together with the nitrogen obtained from nitrate reduction, contributed partly to the protein synthesis and partly to the nitrogen excreted into the water medium. The next period of 25 hours in nitrogen atmosphere resulted in an increase in this nitrogen fraction, which accounted for the slight protein hydrolysis as well as for the nitrogen lost from the sum of amide and ammonia nitrogen.

When the slices were transferred from nitrogen back to air, the "other nitrogen" fraction gained partly from the continued protein hydrolysis and partly from the nitrate and amide nitrogen fractions.

Summary

1. Experiments are reported in which the respiration and the nitrogen metabolism of whole and sliced roots of *Raphanus sativus Aegyptiacus* are

studied. The effect of alternating air and nitrogen atmospheres is also discussed.

2. Respiration of mature whole roots proceeds along a steady low level for prolonged periods. Slicing of the root results in rapid and irregular increase in the respiration rate for about 25 hours, after which the latter subsides until it reaches a steady level parallel to that of the intact root but slightly higher.

3. Transference of radish root slices from air to nitrogen results in a drop in the respiration to a steady level below that in air. On re-exposing the slices to air, their respiration rate increases suddenly to a level higher than the air-line rate and then subsides gradually until it reaches the air-line rate after about 48 hours. The relationship between aerobic and anaerobic respiration rates shows that the oxidative anabolism, which occurs during aerobic respiration, is much lower than that reported by BLACKMAN for apple fruits.

4. Very little change takes place in the various nitrogen fractions in whole mature radish roots. The high irregular respiration of the sliced roots is always accompanied by protein synthesis at the expense of both nitrate and "other nitrogen" fractions. Some soluble organic nitrogen is always recovered from the water in which the slices are floated, and its origin is discussed.

5. Under anaerobic conditions, nitrate reduction is inhibited.

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NITROGEN METABOLISM OF DETACHED CORN LEAVES IN DARKNESS AND IN LIGHT[1]

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(WITH SIX FIGURES)

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When corn seedlings absorb large amounts of ammonium nitrogen from complete nutrient solutions high in ammonium, soluble nitrogen compounds accumulate in the tissues of shoot and root but the true protein remains relatively constant (**15**). Ammonia does not accumulate rapidly in tissues of the corn plant until asparagine, glutamine, α-amino nitrogen and peptides have reached relatively high values. In order to determine the role of these compounds in protein catabolism, the changes in nitrogen composition of detached leaves maintained in darkness and in intermittent light and darkness were studied and the results are reported here.

Yarwood (**20**) has recently reviewed the subject of detached leaf culture. Similar studies have been made on leaves of rhubarb (**14**), barley (**21**), Kikuyu grass (**17, 18**), and sudan grass (**18**), and oats (**17**) cultured in continuous darkness, and tobacco (**12**) and runner bean leaves (**2**) in light and darkness. The results show in general that protein hydrolysis begins soon after the leaf is detached and that amino nitrogen, and either or both of the amides, glutamine and asparagine, accumulate. Whether this loss of protein on detachment of the leaf is due to lack of supply of some external factor or hormone or to some internal factor has been the object of speculation (**1, 16, 13**).

Materials and methods

Seventy vigorous and not fully mature corn leaves and their attached sheaths were selected on July 25 from plants averaging about 3 ft. high in a field of open-pollinated yellow dent corn grown in a fertile field on the North Station Farm. Leaves were taken from the second and third nodes above the soil surface by cutting the sheath just above the node.

Ten leaves selected at random were dried immediately for a control or initial sample; 30 leaves were chosen for a light series and 30 for a dark series. The light series was placed in 6 earthenware 2-gallon crocks (5 leaves per crock) with the leaf sheaths inserted through large holes in the covers. Each crock contained 4 liters of 0.005 N $CaSO_4$ and the leaf sheath dipped 3–4 inches into the solution. The light series was kept in a whitewashed greenhouse with air temperatures varying from 70° to 90° C., and subject to the usual diurnal fluctuations of light intensity. The dark series was similar in all details except that they were kept in a dark room in a temperature range of about 70° to 85° C.

[1] Published with the approval of the Director of the South Dakota Agricultural Experiment Station as Journal Paper no. 208.

At intervals of 3, 6, and 9 days at approximately 3 P.M. ten leaves were chosen at random from the dark series and light series, respectively, for weights and subsequent determinations. Green weights were taken after blotting the excess solution from the sheath, and the leaves dried for 2½ hours in a forced draft oven at 80° C. Glutamine in plant material is stable under these drying conditions (10). Dry weights were obtained and the tissue ground in a Wiley mill to pass a 60 mesh screen. All samples were stored in bottles until analyses could be made.

The analytical procedures used were essentially those used previously (15) except for the following modifications. Ammonia and glutamine- and asparagine-amide nitrogen were determined on extracts made by mixing 3 gm. dried ground tissue and 60 cc. distilled H_2O and heating with stirring for 10 minutes to 75° C., then chilling in a refrigerator and filtering through quantitative paper. Extracts for total soluble and Van Slyke α-amino nitrogen were made by extracting 10 gm. dry tissue with 200 cc. dist. H_2O, heating to 80° C., filtering while hot, and cooling. Three gm. dry trichloracetic acid was added per 100 cc. extract and filtered to give the extract used. Total soluble nitrogen was determined by the iron-reduction method of PUCHER et al. (8). Residual α-amino nitrogen was obtained by subtracting the total amide nitrogen from the Van Slyke α-amino nitrogen (15). Nitrate nitrogen was determined by extracting 2.5 gm. ground dry tissue with 200 cc. hot distilled water in a 250-cc. flask, cooling, clarifying with 10 cc. saturated neutral lead acetate, and filtering after making to volume. To duplicate 100-cc. aliquots of extract and 200 cc. distilled H_2O in a Kjeldahl flask, 25 cc. of 10% NaOH was added and the volume boiled down to about 150 cc. to remove ammonia and hydrolyzable amide nitrogen. When cool, 200 cc. of water and 3 gm. Devarda's alloy were added, the nitrate nitrogen reduced to NH_3-nitrogen and distilled into standard acid.[2] Total nitrogen was obtained by summing the true protein nitrogen and total soluble nitrogen.

Results

The weights and analytical data are presented in table I. The leaves of cultures 4 and 5 at time of harvest were brown and dehydrated along the margins and there was a considerable amount of necrotic tissue at the tips of the blades. In cultures 6 and 7, which were 3 days older, the necrotic areas were more extensive and small brown patches of brown tissue occurred in the blade. A general yellowing of the leaf tissue did not occur, however, in either the dark or light cultures. The pattern of necrosis of leaf tissue was quite similar in both the dark and light series and is comparable to the injury obtained when ammonium nitrogen was supplied to intact plants from relatively concentrated solutions of $(NH_4)_2SO_4$ (15), except that rolling of the leaf blade did not occur.

Necrosis of the leaf tissue may account for the gradual decrease in water

[2] Essentially the method of R. W. GERDEL as modified by O. E. OLSON. Proc. South Dakota Acad. Sci. 20: 95–101. 1940.

TABLE I

WEIGHTS, PERCENTAGE MOISTURE, SUGAR CONTENT AND NITROGEN COMPONENTS OF DETACHED CORN LEAVES WITH SHEATHS CULTURED IN DILUTE $CaSO_4$ IN LIGHT AND IN CONTINUOUS DARKNESS AT THE END OF THE PERIODS INDICATED

	Original	Light			Dark		
Culture No.	1	2	4	6	3	5	7
Days	0	3	6	9	3	6	9
Green Weight, gm.	279	253	261	240	243	251	195
Dry Weight, gm.	33	38	44	47	36	37	37
% Moisture	88	85	83	80	85	85	81
Reducing sugars, % dry wt.	6.25	6.20	7.50	6.04	4.84	3.54	2.01
Sucrose, "	2.77	6.20	3.90	3.96	1.41	1.29	1.11
Total sugars, "	9.02	12.4	11.4	10.0	6.25	4.83	3.12
Ammonia, mgm. N/kg. dry wt.	95	86	101	166	113	267	315
Glutamine, "	82	442	716	776	402	2,066	2,150
Asparagine, "	282	1,018	1,662	2,932	1,818	2,440	2,640
Residual α-amino, "	1,000	2,400	4,000	5,700	4,000	5,100	5,600
Nitrate, "	4,980	4,330	4,300	3,580	4,700	5,980	5,080
Total soluble, "	9,060	11,500	14,700	16,040	13,540	18,600	18,900
"True" protein, "	17,900	13,200	11,100	9,900	13,400	11,750	10,450
Total, "	26,960	24,700	25,800	25,940	26,940	30,350	29,350

content of the leaves under both environmental conditions as shown in table 1. However, dehydration of non-necrotic areas of the leaf may have also been involved. Water content of the leaves may have reached some maximal value between 0 and 3 days that is not shown by the data. VICKERY et al. (**12**) found that detached leaves of tobacco imbibed water from water and glucose solutions and maintained their higher water content for more than 100 hours after detachment.

Although the cultures were not sterile, only culture 7 showed any evidence of gross contamination. In this case the lower one-half inch of the sheath was slimy from bacterial growth. This portion was removed, dried, weighed, and the weight included in the total dry weight. The tissue, however, was discarded.

In the light series, the total sugars and sucrose increased during the period of culture while the reducing sugars remained relatively constant. Detachment of the leaf from the plant apparently blocks translocation of carbohydrate, but does not seriously interfere, if at all, with photosynthesis (**20**). On the other hand, in the dark series, a great loss of both sucrose and reducing sugars occurred. These two series of detached leaves, then represent two distinct levels of carbohydrate supply in comparison to which nitrogen metabolism may be studied.

Due to probable variations in the original weights of the leaves of each culture, the dry weights at harvest (table I) are not reliable indices of changes in weight during the experiment but an increase in weight of the light series due to photosynthesis and a decrease in weight of the dark series due to respiration is quite probable. This inadequacy of the data renders calculations of totals for each constituent of the leaf unreliable.

Nitrogen changes can be evaluated in percentage of total nitrogen, however, if losses of nitrogen did not occur. Losses of gaseous ammonia were not detected as Yemm (**21**) found with detached barley leaves cultured in the dark. At the end of the experiment, all of the solutions for the light series were composited, filtered, and aliquots taken for analyses. The solutions contained a total of 50 mg. total nitrogen of which 92% was NH_3-nitrogen. Composited solutions from the dark series contained a total of 17 mg. nitrogen, but ammonia-nitrogen was not present. No nitrates or nitrites were detected in the residual cultures of either series. The maximum loss of 50 mg. nitrogen for 30 leaves in the light series is equivalent to an average loss of 16.7 mg. nitrogen per 10 leaves. Since the initial leaves contained a total of 890 mg. total nitrogen the loss to the culture amounted to 1.9% of the total nitrogen. This loss is negligible in relation to the total nitrogen and permits comparison of the nitrogen constituents of the different cultures in per cent of total nitrogen per kilogram (dry weight basis), since all values for each culture are subject to the same increase or decrease in the dry weight. There is an increasing amount of evidence that loss of nitrogen in unknown gaseous form may occur (**13**), but it is relatively quite small in relation to the total nitrogen. VICKERY

et al. (12, 14) found that the total nitrogen of detached rhubarb and tobacco leaves did not change within the limits of accuracy of their analytical methods.

Figure 1 shows the changes in nitrogen constituents in per cent of total nitrogen per kilogram dry weight for each culture in the light and dark series, respectively. The distance between two lines represents the percentage for that constitutent of the total nitrogen. In both series hydrolysis of protein nitrogen occurred at a rapid rate and tended to decrease with time so that at the end of 9 days the protein level was little more than half of the initial value. The greater hydrolysis in the dark than in light was consistent and may have been significant, but the oustanding conclusion is that light and a high carbohydrate level resulting from photosynthesis and interrupted translocation exerted no sparing action on the leaf protein. VICKERY et al. (12) obtained similar results with tobacco leaves.

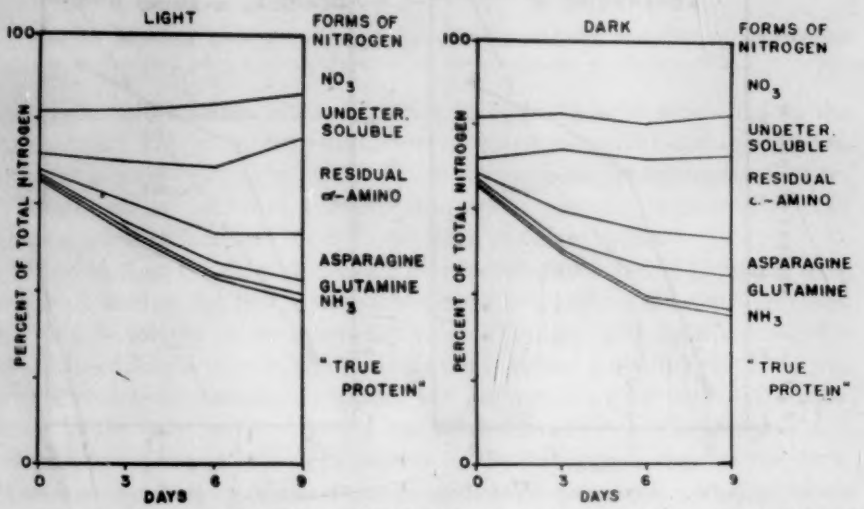
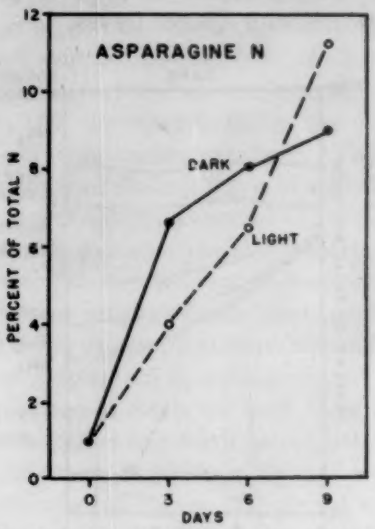


FIG. 1. Nitrogen distribution in detached corn leaves cultured for the period indicated in ordinary daylight and in complete darkness.

Soluble organic nitrogen compounds and ammonia accumulated in the leaf tissues of both series at the expense of the hydrolyzed protein, but some may have come in the light series from the reduction of nitrate and the subsequent combination of the reduction products with keto acids, etc. Amino nitrogen, asparagine, glutamine, and ammonium, in decreasing order of magnitudes, were the forms of nitrogen produced in the dark series. In the light series, undetermined soluble nitrogen accumulated in the early stages of protein hydrolysis, but the other constituents retained their same relative order, as in the dark series. Undetermined soluble nitrogen as here used consists of much peptide nitrogen (15). The relative rank of importance of these compounds or groups of compounds in protein catabolism of the leaf in light is quite comparable to their relative importance in metabolizing ammonia absorbed from the substrate by intact plants (15).

In the light series the accumulation of ammonia in the leaf tissues was less than in the dark series. If it is assumed, however, that the ammonia found in the residual cultures of the light series came from the tissues then calculations show that the amount of ammonium produced is not greatly different. In these experiments, like those previously reported on intact plants, there is no conclusive proof that accumulation of ammonia is responsible for the tissue injury noted since its accumulation is always associated with other profound metabolic changes.

Reduction of nitrates in the leaves of the light series may have occurred as indicated in figure 1. The original leaves were extremely high in nitrates, but this is not unusual for corn growing on soils in this section. The nitrates are not uniformly distributed in the leaf, but are higher in the sheath and the



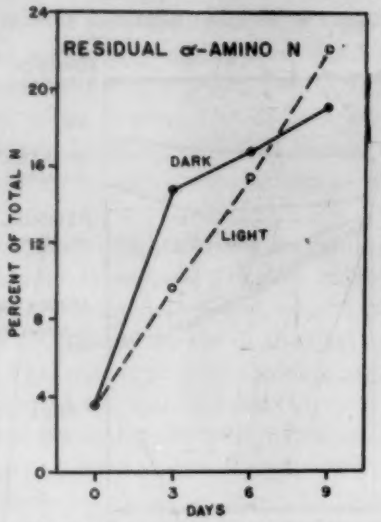


FIG. 2 (left). Asparagine accumulates rapidly in detached corn leaves cultured in either darkness or light.

FIG. 3 (right). Amino nitrogen, in addition to that associated with the amides, accumulates in detached corn leaves and at a rate twice that of asparagine nitrogen.

midrib than in the lamina. At the time of cutting, the leaf blade had a higher total nitrogen content, than the sheath tissue, about twice the α-amino and basic nitrogen content, approximately the same amounts of ammonia, glutamine, and asparagine and about one-half as much water-soluble nitrogen. In the dark series the nitrate content is somewhat erratic and in the interval 3 to 6 days the data indicate that nitrate actually accumulated. Whether this is significant cannot be determined from the data, but nitrate production has been noted in detached leaves cultured in the dark (**5, 12**).

Asparagine nitrogen and residual α-amino nitrogen in per cent of total nitrogen are plotted respectively in figures 2 and 3. Different units for the percentage scale are chosen in the graphs to show the similarity of the

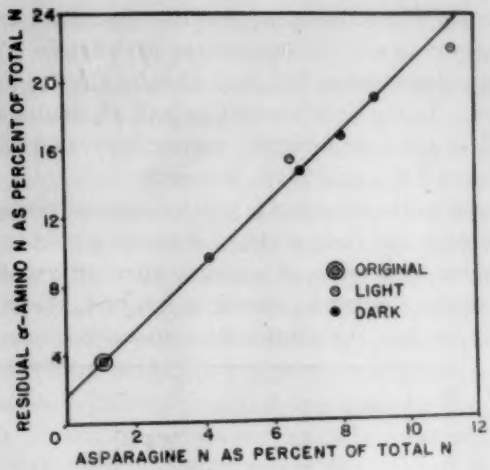


FIG. 4. Residual α-amino nitrogen accumulates at twice the rate of asparagine nitrogen in detached corn leaves regardless of the conditions of illumination.

asparagine and α-amino nitrogen curves in both the light series and in the dark series. This apparent equilibrium of asparagine and α-amino nitrogen, with the α-amino nitrogen twice that of the asparagine nitrogen, is more strikingly shown in figure 4 where asparagine nitrogen is plotted against residual α-amino nitrogen for both the dark and light series.

Figures 2 and 3 show that more asparagine and α-amino nitrogen were produced during the first 6 days of culture in darkness than in light and this may be related to the somewhat greater protein hydrolysis during this period in darkness than in light and to the fact that protein hydrolysis was more complete in darkness, peptides not accumulating as they did temporarily in the light series. At the end of 9 days, however, asparagine and α-amino nitrogen contents were greater in the light series than in the dark. This larger production of these constituents in the interval 6 to 9 days in the light series, as contrasted with the dark series, coincided with a significant decrease in the undetermined soluble or peptide nitrogen of the leaves of the light series.

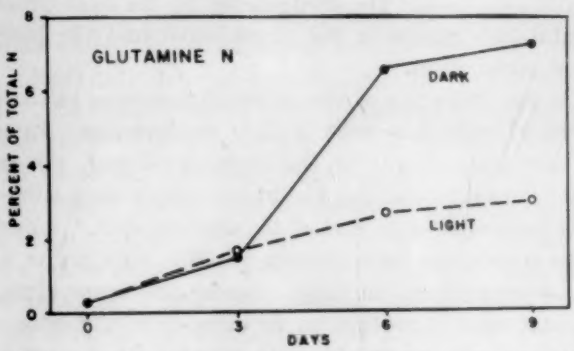


FIG. 5. Glutamine synthesis is favored by darkness in the later stages of culture of detached leaves.

Accumulation of glutamine is quite unlike that of asparagine and α-amino nitrogen as shown by figure 5 in comparison with figures 2 and 3. Glutamine production is much greater in the dark than in the light except in the first 3 days of culture. In the light asparagine and glutamine nitrogen increased 10.3% and 2.7% of the total nitrogen, respectively, in 9 days, whereas in the dark the gains were 8.0% and 7.0%, respectively.

In figure 6 glutamine nitrogen is plotted against residual α-amino nitrogen for both the dark and light series. Whereas a simple equilibrium exists between asparagine and residual α-amino nitrogen regardless of the light conditions and sugar content as shown in figure 4, the experimental conditions profoundly modify the glutamine α-amino nitrogen relationship. In

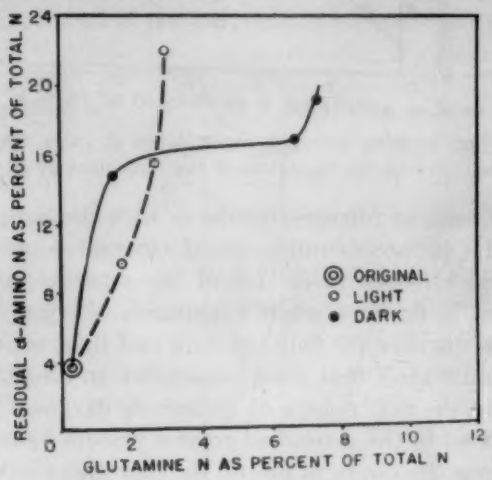


FIG. 6. Glutamine synthesis, unlike asparagine, is not related to residual amino nitrogen accumulation. Compare figure 4.

the light glutamine is unable to keep abreast of α-amino nitrogen accumulation, but in the dark from 6 to 9 days glutamine is preferentially formed.

Discussion

Three significant points are brought out by the data presented that are of fundamental importance to the physiologist and the biochemist in the study of plant metabolism.

The first is the similarity of the catabolic processes in the leaf following its detachment whether it is kept in light or darkness. In spite of an increasing carbohydrate supply in the illuminated leaf, the rate of protein hydrolysis and the accumulation of soluble nitrogen compounds, amino acids, and amides is quite similar to that of the starving leaf. Although detached leaves of some plants can form protein (**7, 16**), VICKERY et al. (**12**) found that tobacco leaves cultured in water, glucose and ammonium solutions lost protein at similar rates in continuous darkness or in continuous light. CHIBNALL (**2**) obtained similar results with cultures of runner bean leaves in

strongly diffused daylight and in darkness. He (1) later postulated hormonic control by the roots of the protein level of the leaf, but reserved judgment until after further experimentation. WOOD (16) has recently discussed hormonic control and VICKERY et al. (13) have recently summarized the problem as follows: "Thus detached leaf culture experiments furnish a picture of the disintegration of the chemical systems of the cell when the controls upon these delicately adjusted relationships are removed. The inference is unavoidable that these controls play their roles effectively only when the organism is intact; accordingly, certain factors in the controlling mechanisms must have their origins in other tissues than the leaves."

PETRIE (6) regarded the protein level as being maintained at a superoptimum concentration by oxidative metabolism with ample carbohydrate, oxygen supply and assimilable nitrogen being essential for the continuous supply of energy. If this picture is correct it is apparent with detached corn leaves that detachment has interrupted the supply of some essential component. To say that it is hormonic is only a convenience.

Studies with heavy nitrogen (4, 11) have shown that the protein of the leaf is constantly undergoing hydrolysis and resynthesis. WOOD et al. (18) have shown that some amino acids are preferentially oxidized in leaves. Such preferential oxidation of amino acids in the protein cycle might lead to a deficiency of an amino acid if it is synthesized only in the roots and translocated to the leaves. The importance of the roots of corn in the synthesis of asparagine, glutamine and α-amino acids has been shown by the writers (15). Further work with detached leaves cultured in dilute solutions of single amino acids, vitamins, hormones, purines, pyrimidines, and extracts of roots is needed.

The second point is the equilibrium existing between asparagine and residual α-amino nitrogen over a wide range of concentrations and independent of the conditions of hydrolysis as shown in figure 4. Such an equivalence either in detached leaves or with intact plants has not been reported. A change of 2% in residual amino nitrogen is equivalent to a change of 1% in asparagine nitrogen.

CHIBNALL and NOLAN (3) have reported that leaf cytoplasmic protein contains 7.44% amide nitrogen. Using this figure, the amount of amide nitrogen expected from the hydrolysis of protein corrected for changes in undetermined soluble nitrogen can be calculated and are shown in table II. This assumes that the undetermined soluble nitrogen is largely peptide (15) and of the same amino-acid composition as the hydrolyzed protein. The equation used is: expected Δ-amide = 0.0744 (loss of protein + loss undetermined soluble nitrogen). Table II also shows the actual changes in amide nitrogen for various periods. Only about one-third of the amide nitrogen synthesized can come from the protein hydrolyzed; the remainder is synthesized in secondary reactions. Other investigations (12, 14, 21) have shown with detached leaves that a large portion of the amides are of secondary origin.

If the amides were of primary origin then a constant ratio of asparagine to residual α-amino nitrogen might be expected. However, since a large part of the asparagine is the product of oxidations of amino acids to produce ammonium, and the combination of this ammonia with aspartic acid, produced possibly in part by transaminations (**9**), the equivalence of amino nitrogen and asparagine cannot be explained with the data now available. This is more significant in view of the fact that probably not all of the amino acids represented in the determined complex enter into the equilibrium. Some are more easily oxidized than others (**18**), and some do not enter into transamination reactions (**9**).

The third point is the effect of darkness on the accumulation of glutamine. In the light cultures the rate of accumulation of glutamine decreased with time and did not keep pace with the accumulation of residual amino acids. In darkness, however, glutamine synthesis was very rapid in the 3–6

TABLE II

CALCULATED INCREASE IN AMIDE NITROGEN EXPECTED FROM PROTEIN AND PEPTIDES HYDROLYZED, THE INCREASE IN AMIDE OBTAINED, AND THEIR RATIO

PERIOD	AMIDE NITROGEN		
	CALCULATED	OBTAINED	OBTAINED/EXPECTED
Light	*% of total N*	*% of total N*	
0–3 days	0.72	2.28	3.2
0–6 days	1.33	3.94	2.96
0–9 days	2.01	6.48	3.22
Dark			
0–3 days	1.27	3.45	2.72
0–6 days	2.11	6.79	3.22
0–9 days	3.22	7.48	2.32

day period and at the end of 9 days they contained more than twice as much glutamine as the leaves in the light. Figure 6 shows no relationship between glutamine and residual amino nitrogen. Apparently metabolism conditioned by the presence or absence of light is of primary importance in the secondary synthesis of glutamine.

VICKERY et al. (**12**) found that light was essential to glutamine synthesis in cultured tobacco leaves, almost none being formed in leaves in the dark. Although these results with corn are contradictory to those with tobacco, they are not as clear cut because there was appreciable glutamine synthesis under both conditions.

Adequate explanation of the results obtained here cannot be given until more is known of the intermediates, precursors, and enzyme systems involved in bringing about the observed changes in composition.

Summary

1. Leaves with attached sheaths of field-grown dent corn were cultured in continuous darkness and in a greenhouse for periods up to 9 days, with

the sheaths dipping into dilute $CaSO_4$ solution. At 3-day intervals ten leaves from each series were removed, weighed, dried, reweighed and ground for determinations of sugars and various nitrogen fractions.

2. Reducing sugars and sucrose were rapidly depleted in the dark, but sucrose increased in the light series while reducing sugars were relatively constant.

3. In percentage of the total nitrogen, protein was hydrolyzed at comparable rates in both series. Residual α-amino acids, asparagine, glutamine and ammonia, in decreasing order of magnitude, accumulated in the leaves. In the early stages of protein hydrolysis in the light, undetermined soluble nitrogen, mostly peptides, accumulated, but was subsequently hydrolyzed.

4. Nitrates were reduced in the light series, but in the dark the results were erratic suggesting that nitrate formation might have been involved.

5. A linear relation between residual α-amino nitrogen and asparagine nitrogen in the leaves in both series was obtained in the ratio 2:1.

6. Glutamine synthesis in the dark was much greater than in the light, and was not related to the amino acid level.

7. The similarity of the metabolic processes in light and dark is discussed in connection with the role of external factors in protein regulation in the leaf.

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STUDIES IN THE METABOLISM OF CRASSULACEAN PLANTS: THE BEHAVIOR OF EXCISED LEAVES OF *BRYOPHYLLUM CALYCINUM* DURING CULTURE IN WATER

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(WITH TWELVE FIGURES)

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A fundamental problem in experimental work with living tissues is the selection of samples in such a way that differences in composition which result from the treatment to which the material is exposed shall represent the effects of the treatment alone. There should be no opportunity for other processes to intervene and obscure the results. Complete isolation of the system under study can be effected, in the case of leaf tissue, only by excision from the plant. This has the disadvantage that the leaves are then merely "surviving organs" and soon begin to undergo changes that are ultimately lethal. Nevertheless, for a reasonable length of time, such leaves can be assumed to behave in a manner that reflects the normal course of metabolic events.

The technique of excised leaf culture as applied in this laboratory (**4**, **5**) consists in the selection at random of a group of approximately equal samples from a quantity of leaves picked from the plants at the same time, and therefore, presumably, in the same biological and chemical condition. One or more samples are at once prepared for analysis, in order to provide a point of departure for the series, and the others are subjected to treatment. Samples are subsequently withdrawn from time to time for analysis, the data obtained being computed in terms of equal quantities of fresh tissue as weighed at the start of the experiment. Biological variation is minimized by the use of moderately large numbers of leaves per sample. Although the results are expressed in concentration units, they refer to originally equal and similar lots of tissue. Accordingly, the differences from sample to sample furnish, within the limitations of biological and statistical variation, a measure of the actual magnitudes of the changes that have occurred. Errors arising from translocation are eliminated and, presumably, the only phenomena that affect the data are those associated with photosynthesis, respiration, absorption of culture solution, transpiration, and chemical transformations occurring within the cells.

The diurnal variation of acidity characteristic of crassulacean plants is a phenomenon admirably suited for study by this general method. The entire cycle of changes is normally completed within 24 hours, a period sufficiently short to justify the assumption that irreversible catabolic changes which may be initiated are of minor significance in comparison with the

relatively much larger chemical changes associated with the normal metabolism of organic acids and carbohydrates. Accordingly, in the present paper, three experiments are described in which excised leaves of *Bryophyllum calycinum* were subjected to culture in water. In the first, leaves collected in the afternoon, at the time of low acidity, were cultured for 24 hours in the greenhouse exposed to the normal variation in illumination; in the second, similar leaves were cultured in complete darkness for 2 days and, in the third, leaves collected in the early morning, at the time of high acidity, were cultured in complete darkness also for 2 days.

Methods

Preparation of samples

On March 12, 1940, 9 Bryophyllum plants from the lot described in a previous paper (**3**) were selected, and the compound leaves, beginning at the second from the top, were removed from 5 of them at 4 P.M. The individual leaflets were cut from the petioles and rapidly sorted into a number of piles according to size. The samples for the experiment were then chosen so that the same number of leaflets from each pile was included in each, there being three samples of 33 leaflets taken for one experiment and four samples of 34 leaflets for the second. One sample from each group was immediately placed in the drying oven at 80° C.

The samples to be subjected to culture were arranged in long V-shaped troughs with the bases of the leaflets immersed in water, the blades being supported without overlap by the walls of the troughs. The troughs containing the 33-leaflet samples were placed in the greenhouse, the others in a completely dark room. All samples were in position within half an hour. The temperature of the greenhouse ranged from 20 to 27° C. during the 24-hour period of the experiment, that of the dark room from 21 to 23°.

The following morning at 7, shortly after sunrise, the other 4 plants were treated in the same way, four 39-leaflet samples being taken of which three were set up in the dark room by 7:15, the fourth being at once dried. The experiment carried out in the greenhouse was continued for 24 hours; the two experiments in darkness were extended for a second day in order to obtain information on the initiation of catabolic changes.

The process of selection of samples provided for randomization within each sample with respect to the plant of origin. The plants chosen for the afternoon samples corresponded in size with the larger plants described in a previous paper (**3**), those chosen for the morning samples corresponded to the smaller plants. Fundamental data on all of the samples are shown in table I. Analytical results obtained as percentages of the crude dry weight were calculated, with the use of factors derived from these figures, so as to give the quantity of the component that would have been obtained if each sample of fresh leaf had weighed exactly 1000 grams at the start.

The limits within which the samples duplicated each other with respect to composition may best be illustrated by the data for total nitrogen and ash.

Regardless of metabolic transformations within the cells during the period of culture, there should be no significant loss of nitrogen nor of inorganic components from the samples and no gain from the water used as culture medium. The mean value for the total nitrogen of the 11 samples was 1.747 ± 0.072 gms. per kilo of original fresh weight. The standard deviation is ± 4.1% of the mean. The mean value for the ash was 8.94 ± 0.020 gm. per kilo, the deviation being ± 2.3% of the mean. Accordingly, it may be assumed that differences substantially greater than ± 4% observed for other components represent significant alterations in composition as a result of the treatment.

TABLE I

FUNDAMENTAL DATA ON SAMPLES OF EXCISED *Bryophyllum calycinum* LEAFLETS SUBJECTED TO CULTURE IN WATER. FIGURES NOT OTHERWISE DESIGNATED ARE GRAMS. THE TIMES ARE STANDARD TIME

TIME OF SAMPLING	ELAPSED TIME	NUMBER OF LEAFLETS	FRESH WEIGHT: START	FRESH WEIGHT: END	CRUDE DRY WEIGHT	CRUDE DRY WEIGHT PER KILO	FRESH WEIGHT PER KILO: END
	hrs.		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
ALTERNATE DARKNESS AND LIGHT							
4 P.M.	0	33	193.1	.....	18.2	94.26	.....
6 A.M.	14.0	33	182.5	185.6	19.0	104.1	1017
4 P.M.	24.0	33	180.0	177.5	18.9	105.0	986
DARKNESS: STARTING IN AFTERNOON							
4:30 P.M.	0	34	231.3	.....	21.3	92.08	.....
6:30 A.M.	14.0	34	217.6	219.9	21.2	97.4	1010
4:30 P.M.	24.0	34	219.8	221.9	21.2	96.45	1010
4 P.M.	47.5	34	202.3	201.5	18.7	92.43	996
DARKNESS: STARTING AT DAYBREAK							
7 A.M.	0	39	249.4	.....	23.7	95.01	.....
4:15 P.M.	9.25	39	243.5	252.3	23.1	94.86	1036
8 A.M.	25.0	39	250.5	257.3	21.7	86.64	1027
8 A.M.	49.0	39	251.1	247.0	21.5	85.61	983.5

Results

CULTURE IN ALTERNATE DARKNESS AND LIGHT

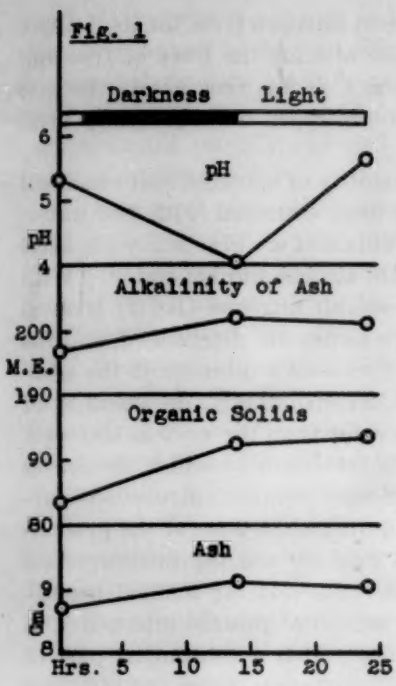
That diurnal variation in acidity occurs in detached leaves of Bryophyllum subjected to culture in water is evident from the curve for pH in figure 1. During the night, the pH dropped by 1.3 units, owing to synthesis of acids, and rose again during the day to a value even higher than that observed initially. The change was about twice as great (in pH units) as that observed in the leaves of intact plants described in a previous paper (**3**). These observations alone are sufficient to justify the fundamental assumption on which the present experiments are based, namely, that excision of the leaves does not seriously interfere with the normal metabolic functions for at least 24 hours.

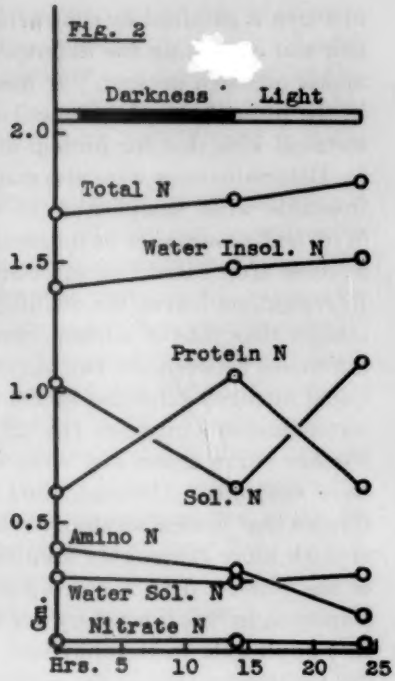
Notwithstanding the large change in total acid reflected in this change in pH, the alkalinity of the ash (fig. 1) remained constant. The mean value for the alkalinity of the ash of the entire set of 11 samples was 195.1 ± 5.8 milliequivalents per kilo, the deviation being ± 3% of the mean. This is the same order of magnitude as the deviation in the nitrogen and ash content and is clearly a measure of the reproducibility of the samples rather than of any effect of treatment. The alkalinity of the ash is a determination of the difference between the number of equivalents of inorganic acidic and of inorganic basic components present after ignition of the tissue; inasmuch as no opportunity was afforded for translocation of inorganic acid or base into or out of the leaves, there was no way in which this difference could be altered by the treatment to which the leaves were subjected.

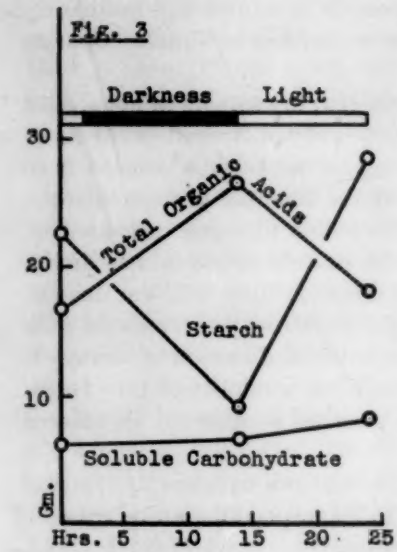
Organic solids apparently increased during the period of darkness and remained essentially constant during the day (fig. 1). However, the analytical determination of organic solids is especially difficult and subject to error and it is by no means certain, in view of the small number of samples, that the apparent increase, although it amounted to about 10%, was real. The similar samples cultured in the dark room (fig. 5) showed no comparable effect. The ash (fig. 1) and the total nitrogen (fig. 2), as has already been pointed out, showed no significant change in the course of the experiment.

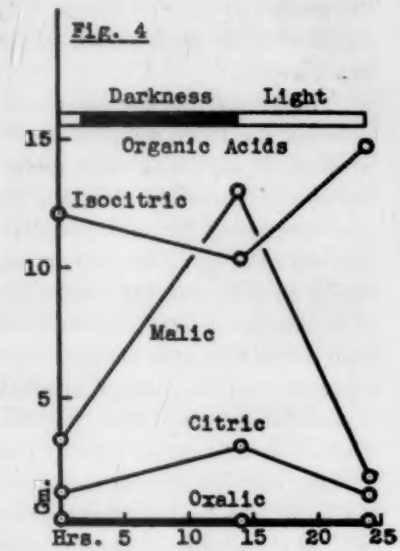
The data presented so far are thus in agreement with the view that the three samples used for this experiment were initially sufficiently alike in composition to warrant the conclusion that changes in excess of 10% with respect to any analytical component represent alterations in composition as a result of the treatment to which they were exposed. The change in protein nitrogen (fig. 2) furnishes an illustration of this; during the night, the protein nitrogen increased from 0.61 to 1.06 gm. per kilo and then diminished during the day to 0.65 gm. The change was similar both in magnitude and in its relationship to light to that observed in leaves of intact plants subjected to analogous treatment (**3**). Furthermore, as in the previous experiment, there were changes in soluble organic nitrogen symmetrical with those of protein nitrogen, the one increasing when the other diminished and *vice versa.*

That the change in organic soluble nitrogen should be roughly equal and opposite to the change in protein nitrogen is to be expected. The quantity denoted protein nitrogen represents the nitrogen of the dried leaf tissue that remains insoluble after a sample is exhaustively extracted with hot 70% alcohol, a procedure designed to remove chlorophyll and other pigments together with the soluble carbohydrates and simpler nitrogenous components, and subsequently further extracted with boiling water to remove a small additional quantity of water-soluble nitrogenous material. It is assumed that nitrogen that remains insoluble under these conditions belongs exclusively to denatured protein. Wide application of the method has hitherto revealed no case that throws doubt on this conclusion. The soluble organic









FIGS. 1 to 4. Composition of excised leaflets of *Bryophyllum calycinum* cultured in water for 24 hours in alternate darkness and light. Data are expressed in grams per kilo of fresh weight.

nitrogen is obtained by subtracting the protein nitrogen from the total nitrogen and correcting the difference for nitrate and for the trace of free ammonia nitrogen present. If these are constant, as was very nearly the case in the present samples, the curve for soluble nitrogen must be nearly symmetrical with that for protein nitrogen.

Determinations were also made of the quantity of nitrogen that remained insoluble when samples of the dry tissue were extracted with hot water. With leaf tissues such as tobacco, results so obtained are practically the same as those after extraction successively with hot alcohol and hot water. With Bryophyllum leaves, the quantity of water-soluble nitrogen (fig. 2) is much smaller than that of soluble organic nitrogen as usually determined, and the difference between the two curves (fig. 2) furnishes a measure of the additional nitrogen extracted by hot alcohol. Correspondingly, the quantity of water-insoluble nitrogen (fig. 2) is much greater than the protein nitrogen. Neither curve shows any clear effect of the treatment to which the leaves were subjected. Bryophyllum leaves obviously contain nitrogenous substances that possess solubility relationships quite unlike those of the proteins of such more thoroughly studied leaves as tobacco, and the interpretation of the present data is correspondingly uncertain. If the method usually employed in this laboratory for the determination of protein nitrogen is in fact applicable to Bryophyllum, the data imply that a substantial part of the protein present in the early morning is converted, under the influence of light, into products that are soluble in hot alcohol. During the night, these products are reconverted into material, presumably protein, that has the same solubility as before. That the alteration in solubility is not merely an effect of the changed pH of the leaves was established by suitable separate experiments.

The question of the nature of the products that are soluble in hot alcohol remains for more detailed study. That they are not normal products of proteolytic action such as amino acids or simple peptides is evident from the curve for amino nitrogen (fig. 2) for, at the time the protein nitrogen was increasing by substantially 0.4 gm., the amino nitrogen decreased by less than 0.1 gm.; correspondingly, while the protein nitrogen was diminishing by 0.35 gm. the amino nitrogen also diminished by 0.17 gm. instead of increasing. The failure of these analytical quantities to correspond with each other either in magnitude or in the anticipated direction of change is evidence that the diurnal alteration in the apparent solubility of the protein is something other than protein digestion such as is observed in tobacco leaves cultured under similar conditions.

Diurnal variation of the total organic acids is shown in figure 3. During the night, the acids increased from 16.9 to 26.7 gm. per kilo, an increase of 58% of the evening value; during the following day, they decreased to 18.1 gm. As has already been shown by the curve for pH, the normal diurnal process of organic acid metabolism characteristic of this species was in no detectable way interfered with by excision of the leaves. Corresponding in

point of time with the change in the acids, there was an even more extensive change, although in the opposite sense, in the quantity of starch. Starch decreased during the night from 22.6 to 9.2 gm. per kilo (calculated as glucose) and increased during the day to 28.5 gm. Nevertheless, the soluble carbohydrates scarcely changed significantly; there was at most a slight increase during the day.

The chemical possibilities presented by these changes in composition are far too complex for detailed interpretation, and all that can be attempted is to point out certain inferences that may justifiably be drawn. The quantities of starch and organic acids involved are such that a roughly quantitative relationship of the nature of an equilibrium between starch and acid might be assumed to exist, that is to say, a reaction analogous to that offered as a speculation by BENNET-CLARK (1) to account for the chief chemical reactions of crassulacean metabolism. During the night, 13.4 gm. of starch disappeared while 9.8 gm. of organic acids were formed. There was no change in the soluble carbohydrates so that the net change represents a loss of 3.6 gm. of organic substance. No such loss appears on the curve for organic solids; rather, there was an apparent increase of about 9 gm. which, from other considerations, it seems more conservative at present to interpret as being possibly due to biological variation between samples. Thus the evidence, as far as it goes, suggests that the respective alterations in the quantities of acids and of starch were the result of intracellular chemical reactions and it is quite possible that the acids arose in the course of a series of oxidation reactions which consumed starch.

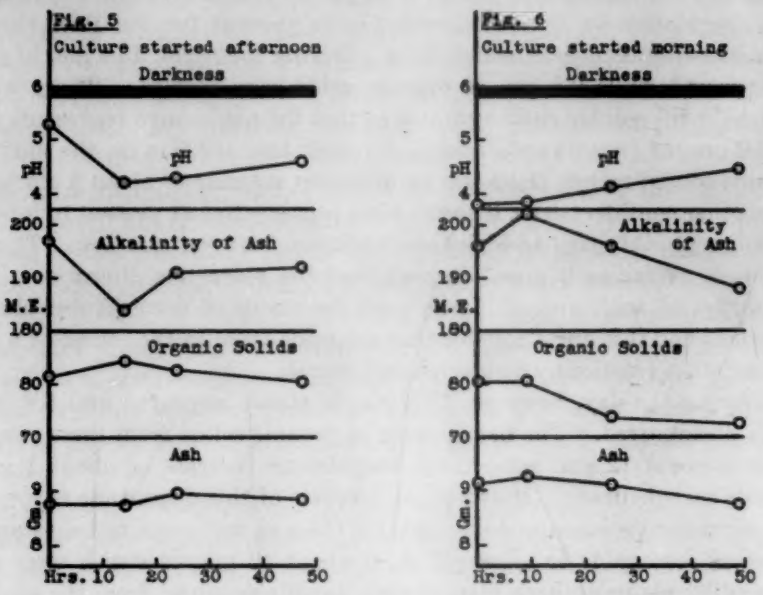
During the day, however, 19.3 gm. of starch appeared and 8.6 gm. of acids disappeared. The net increase in organic solids from these two reactions is about 12 gm. since there was also an increase of about 1 gm. of soluble carbohydrate. However, no increase of this magnitude is shown by the curve for the organic solids (fig. 1); these in fact remained constant and it seems reasonable to assume that, if about 12 gm. of starch were newly formed by photosynthesis from carbon dioxide acquired from the air, there would have been a clearly evident rise of the curve in figure 1. Thus the formation of starch in light must, in this case, have also been largely the result of intracellular reactions and the quantity is considerably greater than could possibly have arisen from the organic acids even on the most favorable assumptions as to the efficiency of the reversal of the equilibrium which may have operated during the night.

By far the greater part of the change in total organic acids arose from the change in malic acid (fig. 4). This substance increased from 3.3 to 13.0 gm. per kilo, or by a factor of 4 during the night and decreased to less than the original value during the day. Citric acid underwent a parallel series of changes increasing from 1.3 to 3.1 gm. per kilo during the night and falling again during the day to 1.2 gm. Isocitric acid, however, behaved quite differently; it decreased from 12.1 to 10.4 gm. per kilo during the night and increased to 14.8 gm. during the day. These changes are in the opposite

sense to those of the malic and citric acids and they are proportionally much smaller ones; isocitric acid does not respond to illumination as extensively as the other two acids. The small quantity of oxalic acid present showed no significant change as a result of the treatment of the samples.

Culture in complete darkness

The data for the experiments carried out for 2 days in complete darkness are plotted in separate figures placed side by side for more convenient comparison of the effects upon leaves picked in the one case in the afternoon at the time of low acidity and, in the other, in the morning at the time of high acidity, these leaves, of course, having been in darkness during the night.

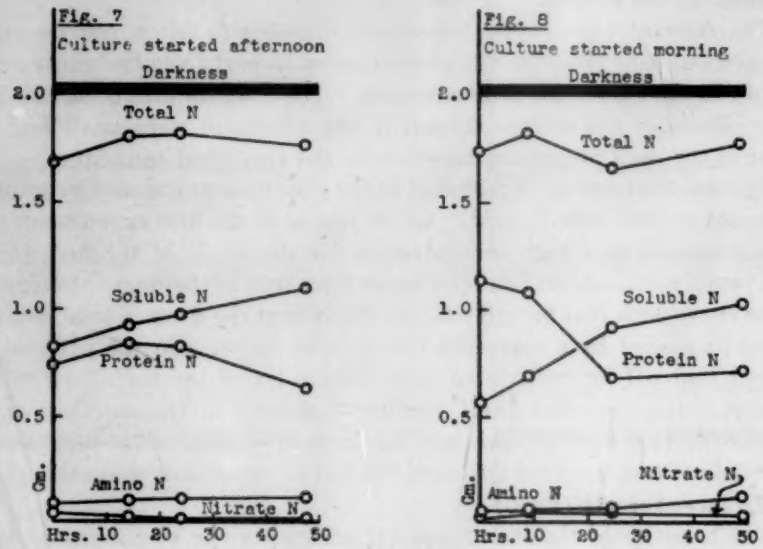


Figs. 5, 6. Composition of excised leaflets of *Bryophyllum calycinum* cultured in water for 2 days in complete darkness. Data are expressed in grams per kilo of fresh weight. Figure 5 refers to leaves started in the afternoon at a time of low acidity; figure 6 to leaves started in the morning at a time of high acidity.

The pH of the samples placed on experiment in the afternoon was high (fig. 5) and dropped for the first few hours in the same way as it did in the first experiment. It then began slowly to rise and had increased by 0.4 units at the end of 2 days. The pH of the samples started in the morning was low (fig. 6) and there was a slow and steady rise over the 2-day period that totaled about 0.6 units. The early parts of these curves reflect metabolic changes that were probably not greatly different from those that occur in normal leaves; the events that took place during the second day, however, doubtless reflect the behavior of cells placed under gradually mounting metabolic stress. One of the first demonstrable effects of this stress is clearly a diminution in the acidity of the leaves.

The curves for alkalinity of ash, organic solids, and ash (figs. 5 and 6) and for total nitrogen (figs. 7 and 8) show, as did the similar data for the first experiment, no significant effect of the treatment of the samples. It is quite likely that the small drop in organic solids at the end of these two experiments represents a loss of organic material from respiration but the samples were too few in number and the experiment was not sufficiently prolonged to make this certain. Analogous experiments with tobacco leaves (4) and rhubarb leaves (5), however, have shown that such a result is to be anticipated.

The behavior of protein nitrogen in the two experiments was entirely different (figs. 7 and 8); in the samples started in the afternoon at a low



FIGS. 7, 8. Composition of excised leaflets of *Bryophyllum calycinum* cultured in water for 2 days in complete darkness. Data are expressed in grams per kilo of fresh weight. Figure 7 refers to leaves started in the afternoon at a time of low acidity; figure 8 to leaves started in the morning at a time of high acidity.

level, protein at first increased. After passing through a flat maximum, the quantity of protein nitrogen then decreased during the second day of the experiment. However, neither change was as striking as those encountered in the first experiment (fig. 2); the increase was about 13% of the afternoon value and was thus probably significant, the decrease was 25% of the maximum value and of the validity of this there can be little doubt.

The samples started in the morning at a high level of protein decreased only slightly in protein content during the first 9 hours but had dropped by 39% at the end of 24 hours. There was no significant change during the second day. The effect of culture in darkness on the protein of Bryophyllum leaves is thus dependent upon the level of the protein present in the

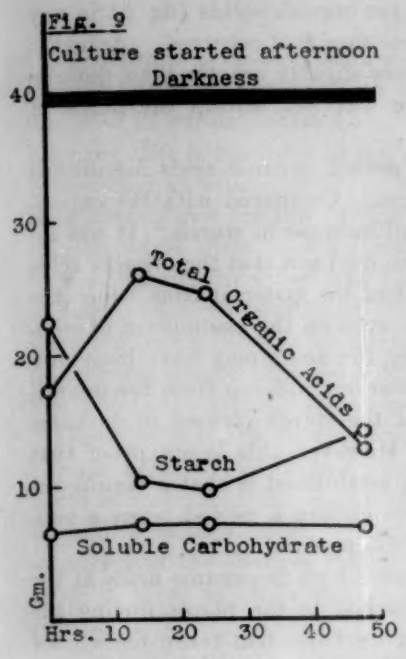
leaves at the beginning of the experiment. If the culture is started at a low level at the end of the day, there appears to be a small increase, but if it is started in the morning at a high level, there is little if any significant change for about 9 hours; then, however, a change analogous to that observed in light supervenes and there is a marked fall. Interpretation of these changes must await more detailed analytical studies; for the present, all that can be stated is that the fall in protein is not accompanied by a marked rise in amino nitrogen (fig. 8) as would be expected if the reaction were one of protein hydrolysis. Rather, it would appear to be one of change in solubility of a substantial part of the protein such as might be supposed to result from some form of disaggregation into units that are not rendered insoluble by hot alcohol.

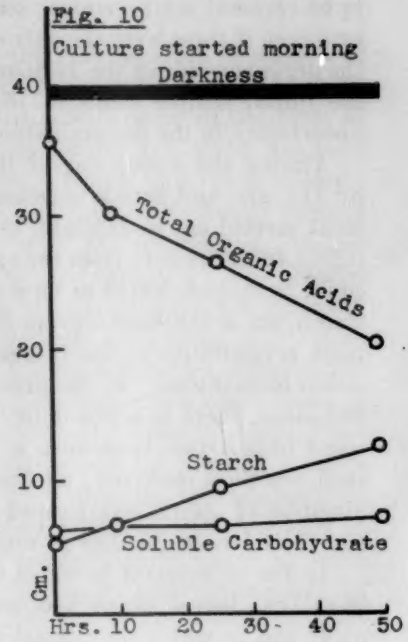
The failure of the curves for soluble nitrogen to follow courses exactly symmetrical with those for the protein arises in part from the minor irregularities in the curves for total nitrogen. The changes that occurred in the early phases of the culture started in the afternoon were small and were about at the limit of certain detection by the analytical techniques.

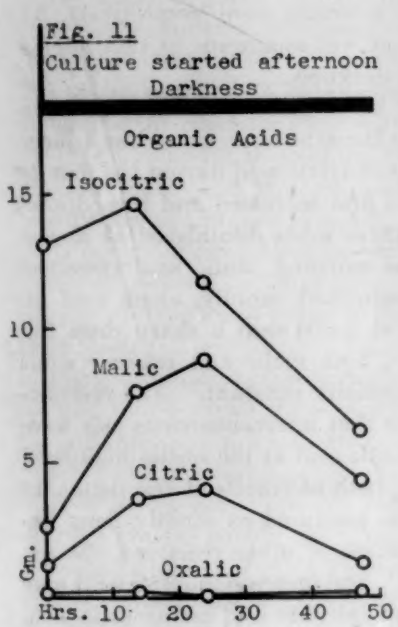
The leaves placed on experiment in the afternoon at low acidity promptly increased in total organic acids (fig. 9) just as in the first experiment. The acids remained at a high concentration for the whole of the first day but then rapidly diminished to a level lower than that at the start. Comparison of the curve with that for pH (fig. 5) shows that the drop in total acids was not accompanied by a correspondingly large increase in the pH and it is evident that pH by itself is an unreliable index of the magnitude of such changes. On the other hand, the leaves started in the morning at high acidity (fig. 10) did not maintain the level of organic acids then present; a continuous loss occurred although this did not become immediately evident on the curve for pH (fig. 6).

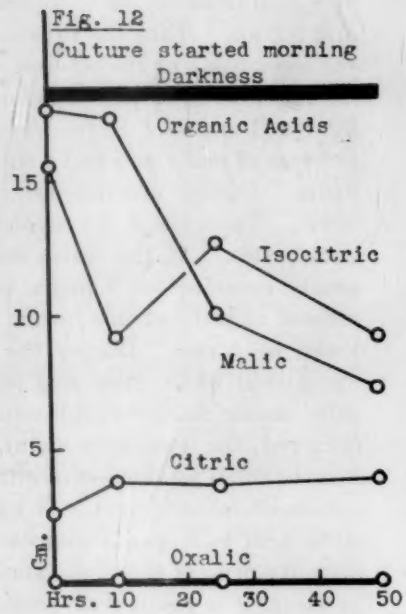
The behavior of the starch was surprising; in the leaves started in the afternoon (fig. 9), starch decreased during the period that the acids were increasing, remained constant while the acids were maintained at a high level but then *increased* during the period that the acids were falling in concentration. This represents synthesis of starch in leaves in total darkness, an unusual observation at best, and one that might well be attributed to error had it not been confirmed by an even more striking synthesis of starch in the leaves started in the morning (fig. 10). In these, starch increased from 6.2 to 12.6 gm. over the entire 2-day period at a rate which followed a smooth curve essentially symmetrical with that for the total acids.

It will be useful to consider the relative quantities of starch and organic acids involved in these changes. Figure 9 shows that, during the first 14 hours in culture in darkness, 13.4 gm. of starch disappeared while 8.9 gm. of organic acids were formed. The change in soluble carbohydrate was insignificant being only 0.5 gm.; thus it is possible that the organic acids that were formed arose from oxidation reactions in which starch was consumed. There was a net decrease of 4.5 gm. of organic material, too little









FIGS. 9 to 12. Composition of excised leaflets of *Bryophyllum calycinum* cultured in water for 2 days in complete darkness. Data are expressed in grams per kilo of fresh weight. Figures 9 and 11 refer to leaves started in the afternoon at a time of low acidity; figures 10 and 12 to leaves started in the morning at a time of high acidity.

to be revealed with certainty on the curve for organic solids (fig. 5) in any case even if these were the only chemical reactions that occurred. Actually, the organic solids in the 14-hour sample were slightly greater than those in the initial sample although the difference was well within the limits of uncertainty in the determination.

During the second day of the culture period, organic acids diminished by 11.7 gm. and starch increased by 4.3 gm. Compared with the experiment carried out in daylight, this is a small increase in starch. It was inferred from the data from the experiment in daylight that the organic acids could have contributed at most only a part of the material from which the starch was synthesized during illumination even on the assumption of complete reversibility of the reactions whereby the acids may have been produced in darkness. In the present case, however, judging from the quantities alone, there is a possibility that all of the starch formed in darkness could have arisen from such a reversal. However, this is not proof that such reactions occurred; all that has been established is that a significant quantity of starch was formed in darkness during a period when a substantially larger quantity of organic acids disappeared.

In the experiment in which the leaves were high in organic acids at the start (i.e., leaves which had remained attached to the plants during the night and in which the normal reactions of darkness had taken place), 9.4 gm. of acids disappeared during the first day and 6.1 during the second. The quantities of starch formed in these intervals were, respectively, 3.1 and 3.2 gm. Thus the behavior throughout was analogous to that during the second day of the other experiment in darkness.

The details of the transformations of the organic acids are shown in figures 11 and 12. In the leaves started in the afternoon, there was a large increase of malic acid and a substantial one of citric acid during the first 24 hours. During this interval, isocitric acid first increased and then diminished. Throughout the second day, all three acids diminished at almost equal rates. In the leaves started in the morning, malic acid remained nearly constant for 9 hours and then diminished rapidly, citric acid increased slightly at first, while isocitric acid underwent a sharp drop followed by a rise. During the second day, both malic and isocitric acids diminished while citric acid remained essentially constant. The relationships among the three acids strongly suggest that interconversions may have occurred; the temporary maintenance of malic acid at the initial high level and the small production of citric acid may both be results of reactions that consumed isocitric acid. If malic acid was produced as rapidly from isocitric acid as it was decomposed in the course of other reactions, the net quantity present would remain unchanged. The increase in citric acid may be assumed to result from transformation of isocitric acid *via* aconitic acid, a reaction well known to occur in muscle tissue (**2**). In the interval between the ninth and twenty-fifth hours of the culture period, the sharp fall in malic acid and the corresponding increase in isocitric acid may have been the effect of a reversal of the reaction that occurred during the first 9 hours.

Discussion

The chemical changes that occur during the first 24 hours of culture of excised leaves of Bryophyllum are, qualitatively, closely similar to those observed in normal leaves (**3**). Marked fluctuations occur in the form of nitrogen that is insoluble in hot alcohol and hot water and which has been interpreted as representing protein nitrogen. Leaves that have recently been illuminated are low in this component but protein is rapidly formed in such leaves when they are placed in the dark. Corresponding changes in the opposite direction take place in the soluble nitrogen. The acidity, whether measured as pH or by the titration of the organic acids extracted from the tissue, likewise undergoes wide variations, acids being synthesized in darkness and decomposed in light. Starch is formed during illumination but, unless the organic acids are undergoing decomposition, disappears in darkness so that the fluctuations are in the opposite sense to those of the organic acids. The largest share in the changes in the organic acids is taken by malic acid; citric acid varies in the same direction as malic acid but through a smaller range. Isocitric acid, however, varies in a less regular manner and the changes that it undergoes are frequently in the opposite sense to those of malic acid, sometimes being large, sometimes small, in relation to it.

The chief technical advantage of the leaf culture method is that, because of the complete isolation of the biological system, inferences may be drawn from the quantities of substances involved in the reactions provided the changes are large enough to be outside the range of probable biological variation among samples. Within certain limits, therefore, specific interpretations of some of the reactions that occur may be attempted.

It will be useful to point out the contrasts between the behavior of Bryophyllum leaves and those of tobacco when subjected to culture. Excised tobacco leaves cultured in water in light (**4**) exhibit a prompt and surprisingly large increase in organic solids, for the most part because of the synthesis of soluble carbohydrates and starch. In darkness, they undergo equally prompt losses owing to the uncompensated effect of respiration, and these losses fall heavily upon the carbohydrates and malic acid. The protein nitrogen diminishes rapidly under both conditions of culture; evidence was secured that implied the complete destruction of that part of the protein that disappeared, and indicated that the simple nitrogenous products formed underwent a succession of complex transformations. The experiments with tobacco leaves were extended for several days so that there is little doubt that the greater part of the changes observed, especially in the later stages, were catabolic in nature. They showed, however, that leaf cells are the seat of intense metabolic activity, and many of the changes were initiated within the first 24 hours.

Excised Bryophyllum leaves likewise undergo profound metabolic changes in composition during culture but, in this species, some of the more conspicuous changes are a part of the normal cyclic behavior of the tissue

under the influence of diurnal variation in illumination. Certain of the reactions so clearly observed in tobacco leaves were, on the other hand, scarcely if at all demonstrable in this species in part because of the brevity of the experiment. This statement applies in particular to the reactions between the tissues and the oxygen and carbon dioxide of the air; no conclusive evidence was secured of photosynthesis in terms of an increase of organic solids in light, nor could loss of solids by respiration in darkness be demonstrated although it is possible that experiments more prolonged than the present ones might be successful in this. The evidence, as far as it goes, suggests what may be termed a more closed metabolic economy than that of the tobacco leaf, and observations that bear on this aspect of the problem are to be found in the literature. The work of BENNET-CLARK (**1**) and of WOLF (**6**) on the respiratory quotient, for example, shows that the liberation of carbon dioxide drops to a very low level shortly after the leaves are placed in darkness at the time that acid production increases in intensity. It is reasonable to infer that carbon dioxide is being utilized within the tissues for the synthesis of organic acids, possibly, for example, of oxalacetic acid from pyruvic acid by the Wood and Werkman reaction. Later, during continued culture in darkness (i.e., after about 12 hours), carbon dioxide liberation by the leaves increases and this occurs at the time when the organic acids pass their maximum and begin to drop in concentration. Its liberation is an evidence of decarboxylation reactions.

BENNET-CLARK likewise observed that oxygen uptake is high during the early phase of acid production but later diminishes. This is in agreement with the assumption that the formation of organic acids is a result of oxidation reactions involving carbohydrates, particularly starch. It is to be noted that both oxidation and carbon dioxide fixation are essential if the organic acid metabolism of Bryophyllum leaves is to be accounted for in terms of the tricarboxylic acid cycle of KREBS. Pyruvic acid may be assumed to arise from the decomposition of hexoses but an increase in length of the three carbon chain of pyruvic acid to four carbon atoms (i.e., conversion to oxalacetic acid) is essential if the subsequent production of malic acid is to be accounted for. Furthermore, oxalacetic acid is also an essential intermediate for the formation of isocitric and citric acids. Regardless, therefore, of the absence of direct information on the presence of the specific enzyme systems necessary for these reactions, one is justified in advancing, as an hypothesis, the view that the transformations of organic acids observed in Bryophyllum leaves are an expression of the activity of such enzyme systems. On this view, the several organic acids are intimately linked with each other by chemical equilibria and transformation of one acid into another, of carbohydrates into organic acids and even the reverse transformation of organic acids to carbohydrates receive a rational explanation. The synthesis of starch in darkness when the concentration of organic acids is high becomes a phenomenon that is to be anticipated. It should be pointed out that all of the analytical data of the present experiments are in agreement with the requirements of this hypothesis.

During the second 24-hour period of the present experiments, however, other phenomena began to make their appearance. The early culture experiments of BENNET-CLARK showed that the organic acids of *Sedum praealtum* leaves passed through a maximum in concentration after about 12 hours in darkness and subsequently declined. At the same time, carbon dioxide production increased. WOLF has made somewhat similar observations on *Bryophyllum calycinum* leaves, and the phenomena are very likely general for the Crassulaceae. In the present experiments, certain reactions were initiated which were the reverse of those noted during the first day. It is probably correct to assume that such reactions represent the beginning of the catabolic processes that inevitably follow excision of the leaf. One of the first to be demonstrable is, as a rule, the transformation of the nitrogen of protein into forms that are soluble and, in particular, an increase in amino nitrogen presumably produced by proteolytic action. These effects are clearly evident in tobacco and rhubarb leaves subjected to culture under analogous conditions (**4, 5**). They are not especially marked in the present case, however, although the culture started in the afternoon does show them to a possibly significant extent (fig. 7).

Another effect well shown by tobacco and rhubarb leaves is the loss of organic solids from respiration but, again, the present experiments were not sufficiently prolonged for this to become unequivocally clear (figs. 5 and 6). However, the disappearance of organic acids, especially malic acid, with a corresponding increase in pH is invariably observed and the present experiments show this especially well. All three of the chief organic acid components decreased sharply during the second day of the experiment started in the afternoon, two of them followed the same course in the experiment started in the morning. This effect may be regarded as an evidence of the increasing metabolic stress to which the excised leaves were subjected. Even in this case, however, the analogy with tobacco and rhubarb leaves is not close. The Bryophyllum leaves became enriched in starch and there was no effect upon the soluble carbohydrates. In tobacco and rhubarb leaves, on the other hand, carbohydrates are early involved in the general picture of catabolism.

Taken together, then, the chemical evidence of the present experiments suggests a general metabolism adapted for survival under unfavorable conditions. The response, rather early in the period of stress, is to lay up a store of starch. It is quite possible that the well-known capacity of excised leaves of Bryophyllum to survive and reproduce by buds which develop at the margins may be the ultimate expression of such a provision.

Summary

Equal samples of leaflets excised from *Bryophyllum calycinum* plants were subjected to culture with the base of each leaflet immersed in water, one set of samples taken in the late afternoon being treated in the greenhouse and a second set in a completely dark room. A third set collected

early in the morning was likewise placed in the dark room. Analytical data were computed in terms of 1 kilogram of fresh tissue weighed at the time of sampling.

There were no significant effects in any case upon the quantity of organic solids nor upon the ash, the alkalinity of the ash, or the total nitrogen; even the soluble carbohydrates failed to change. The samples cultured in the greenhouse increased sharply in organic acids during the night and decreased during the day, the greater part of the change being due to malic acid with citric acid playing a smaller but similar role. Isocitric acid decreased at night and increased during the day.

Starch varied in a manner the converse of organic acids, the fluctuations being in each case through a wider range than those of the acids. Thus it is possible that organic acids synthesized at night arose from oxidation of carbohydrates. However, some source of starch other than the organic acids must be invoked to account for the starch synthesized during the day, yet the failure of the organic solids to increase significantly indicates that photosynthesis played only a small part.

Protein increased during the night and decreased during the day, soluble nitrogen undergoing similar changes in the reverse direction.

The leaves cultured in darkness starting in the afternoon at low acidity increased in malic, citric, and isocitric acids for 14 hours. The increase in malic and citric acids continued for the rest of the first day but isocitric acid then began to decrease. All three acids decreased during the second day.

Starch decreased during the first day in darkness but increased significantly during the second. Protein increased slightly for 14 hours and then decreased slowly.

The leaves cultured in darkness starting in the morning at high acidity began to lose isocitric acid at once. Malic acid dropped slightly but citric acid increased; later malic acid dropped sharply, isocitric acid increased and citric acid remained constant. During the second day, isocitric acid and malic acid decreased, and citric acid remained unchanged.

Starch increased slowly but continuously throughout the 2-day period in darkness. Protein decreased during the first day and remained unchanged during the second.

The changes in organic acids and starch can be largely accounted for if it is assumed that the several substances are linked by enzymatic reactions in a system analogous to the Krebs tricarboxylic acid cycle.

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PLANT GROWTH WITH ARTIFICIAL SOURCES OF RADIANT ENERGY[1]

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(WITH FIVE FIGURES)

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Introduction

In the design of experiments where plants are to be grown under controlled radiation conditions, it is necessary to have information on the relative power efficiency and the type of growth obtainable with various lamp sources, for the proper selection of equipment. It is well known that the growth form of plants is dependent upon the incident irradiance, photoperiod, and the spectral characteristics of the radiant energy (**1, 2, 11, 14, 15, 16**).

Before the introduction of the gaseous discharge lamps, most of the studies were made using the incandescent tungsten filament lamp as a source (**7, 9, 14**). While the growth rate obtained with the bare incandescent lamp frequently was comparable with that often secured under greenhouse conditions when sufficiently high irradiances were used, the plants usually had thin stems and longer internodes than those grown with solar irradiation. With the advent of the modern type gaseous discharge lamps, RODENBURG (**17**), ARTHUR and his co-workers (**1, 2, 3**), and others studied the growth obtainable with these sources.

ARTHUR and STEWART (**1**) observed production of buckwheat plants under incandescent, neon, mercury, and sodium vapor lamps. They found that, with equal irradiances as measured with a Weston illuminometer, the efficiency in production of dry matter decreased in the following order: neon, incandescent, sodium, and mercury. When the radiant energy was calculated on an equal energy basis, they concluded that the actual order of efficiency was sodium, neon, incandescent, and mercury. When sodium alone was tried (**2**), it was found that the plants did not grow well over long periods of time. Supplementing the sodium with two hours per day from an 85-watt capillary mercury vapor lamp, caused an improvement in growth with a number of varieties of plants.

MITCHELL (**12**) grew tomato plants, using a carbon arc and an incandescent filament lamp as sources. He found that total synthesis of dry matter was greater per unit of time under the incandescent lamp when equivalent energies, as balanced with a Weston cell, were used. The plants synthesized more dry matter, although the height was less, under the carbon arc when equal total radiant energies, as measured by a nonselective detector, were used.

[1] Journal Paper no. 272, Purdue University Agricultural Experiment Station.

JOHNSTON (11) grew tomato under varying combinations of water-filtered incandescent and mercury arc radiant energy and concluded from his studies that a greater amount of dry weight is produced with an incandescent lamp when it is enriched with blue from a mercury lamp to the extent of 14% to 51%.

NAYLOR and GERNER (13), ROHRBAUGH (16), and STOUTEMEYER and CLOSE (18) have published data on plants produced under fluorescent lamps, showing that satisfactory growth can be secured with this source. However, no comparative data for the fluorescent with other lamp sources have been published.

The most important radiation characteristics of lamps to be considered for use in growing plants are (1) spectral energy distribution; (2) proportion of input electrical energy dissipated as radiated infrared; and (3) efficiency of production of visible radiant energy within the region of about 4500 A to 7000 A. The proportion of electrical energy dissipated as radiated infrared sets a maximum limit on the visible irradiance which can be used with bare lamps. The infrared beyond 8000 A apparently has little effect other than to raise the temperature of the foliage. If the proportion of radiated energy in the infrared is high, as with the incandescent lamp, excessive heating of the leaves may occur at high irradiances unless suitable filters are interposed between the lamp and the plants.

The present experiments were designed to investigate the comparative growth responses of certain herbaceous annuals under several sources of artificial radiant energy, including the fluorescent, incandescent and high pressure mercury arc lamps. These lamp sources were selected because they represent the most practical types of equipment for general use on the bases of (1) commercial availability; (2) installation cost; (3) ease of lamp replacement and operation; and (4) power efficiency in terms of visible radiant energy production.

Radiation characteristics of lamps

The spectral characteristics and electrical power efficiency of lamp sources depend primarily on the type of radiant energy producing system used; i.e., incandescent filament, gaseous discharge, and/or phosphors. Secondary in importance are operating conditions such as lamp voltage, ambient temperature, age of lamp and type of reflector system. The spectral, electrical, and physical characteristics of commercially available lamp sources are thoroughly discussed in manufacturers' bulletins (8) and in engineering publications on lamp characteristics (4, 5, 6).

The energy distribution of the general service type of *tungsten filament incandescent lamp* is characterized by a continuous spectrum which has a maximum in the near infrared at about 10,000 A. The energy continuously decreases throughout the visible spectrum as the wavelengths become shorter and is practically zero at about 3200 A, which is the ultraviolet transmitting limit of ordinary glass. The incandescent lamp is a source which, compared

to solar radiant energy, is relatively rich in red and weak in blue radiant energy. A 500-watt incandescent lamp radiates beyond the bulb only 12% of the input watts into the visible spectrum and over 70% is radiated as infrared. Most of the remaining 18% is dissipated as heat to the surrounding air and to the base of the lamp by convection and conduction. The ultraviolet radiated beyond the bulb is less than 0.1% of the total input watts.

The luminous efficiency of incandescent lamps of from 500 to 3000 watts in the general service types varies from slightly less than 20 to nearly 30 lumens per watt. The lumen is a convenient unit of luminous flux and evaluates spectrally the radiant energy emitted by the lamp in terms of the sensitivity of the human eye. The footcandle is a similar unit of irradiance. In spite of serious limitations as discussed in a previous report (**19**), the lumen and the footcandle are still the most practical units of visible radiant flux and irradiance respectively. These units are useful, however, only in comparing sources of similar spectral distribution within the visible spectrum.

One of the principal advantages of the incandescent lamp for general use is that no auxiliary equipment is required for its operation. Therefore, the luminous efficiency figures given above represent overall efficiencies. The rated life for general service incandescent lamps in wattages from 500 to 1500 is 1000 hours.

The spectral energy distribution of the *high pressure mercury arc* in glass is characterized by the line spectrum of mercury, with a small proportion of the total radiated energy as a continuous spectrum. Most of the energy within the ultraviolet and visible spectrum is distributed within six groups of lines at 3341, 3654, 4047, 4358, 5461 A and the doublet centered about 5780 A. The mercury arc is one of the most efficient sources of blue radiant energy, but is extremely low in the red. Even for the glass lamps, from 1% to 2.3% of the input wattage is radiated as ultraviolet. For the 400-watt H–1 lamp, which was used in these experiments, the radiated infrared is less than that of the incandescent, but higher than the fluorescent.

The rated luminous efficiency of the 400-watt lamp alone is 40 lumens per watt. When account is taken of the 52 watts dissipated in a single lamp transformer, in addition to the wattage consumed by the lamp itself, the overall luminous efficiency is actually 35.4 lumens per watt. These lamps have a rated life of 3000 hours.

The *fluorescent lamp* is a type of low pressure mercury arc, in which the inside surface of the glass is coated with phosphors which transform the ultraviolet energy of the mercury line at 2537 A into radiant energy of longer wavelengths, which include the visible and ultraviolet. The fluorescent lamp phosphors emit a continuous spectrum, the energy distribution of which depends upon the choice of phosphor materials. The proportion of the input power radiated within the visible spectrum is a function of the nature of the phosphor, and length and diameter of the tube and other design and operating factors. For the 40-watt daylight fluorescent lamp, about

0.8% of the input watts is in the ultraviolet, 20.5% is radiated in the visible, and 26.5% is radiated as infrared. About 53% of the input energy is dissipated in convection and conduction. Thus most of the input energy is carried away to the surrounding air and is not available directly for raising the temperature of the foliage of plants placed under the lamps.

The overall luminous efficiency of the 30-watt white fluorescent lamp, which was used in these experiments, is 40.5 lumens per watt; for the daylight lamp, the efficiency is 37.2 lumens per watt. These figures are based on the use of 115-volt tulamp auxiliaries. The rated life of the fluorescent lamps, standard lamp series, is 2500 hours.

Experimental methods

The plants were grown in air-conditioned growth chambers. The air temperatures were controlled within $\pm 0.5°$ to 3° C. of the temperatures designated in the tables, depending on the lamp units. The humidity was kept in the range of 70% to 80%. Subirrigation gravel culture was used, with a double concentration of a Purdue F nutrient solution (**20**). Manganese was supplied weekly to the solution and iron three times weekly. No other micronutrients were added as previous experience had shown that the gravel was capable of supplying all of the other essential micronutrients. The pH was maintained between 5.5 and 6.5, except for soybean, in which case the range was 6.5 to 7.0. The nutrient solution was changed every two weeks. All of the plants received the same solution, regardless of the radiation treatments. This caused some difficulty because the nutritional requirements were found to be somewhat different for the plants growing under the various lamp sources.

Tungsten, fluorescent, and high pressure mercury arc (H–1) lamps were used as indicated in the tables. The footcandle measurements were made with a Weston photronic cell equipped with a green filter to correct its spectral sensitivity to that of the human eye. The measurements were checked with a Macbeth illuminometer. The footcandle (fc) values given are those at the gravel surface at the beginning of the experimental period. As the plants became taller, the values varied from the original ones given. Calculations for power consumption and total radiant energies were made from data presented by BARNES and his co-workers (**4, 5, 6, 8**). The number of hours of radiation differed in the various experiments and are shown in the tables. The radiation treatments with the sources were varied on the basis of equal power consumption, equivalent footcandles and equal total visible energy.

Results

EQUAL POWER CONSUMPTION

Nobel spinach and Heart of France variety of China aster were grown for 50 days under the radiation treatments designated in table I. The lamp sources were 1000-watt standard incandescent, 3500° K., 30-watt white fluo-

rescent and 400-watt H–1 high pressure mercury arc lamps. The power consumption was the same per square foot for the different sources. Under these conditions, the total visible radiant energy under the incandescent was 58% of that under the fluorescent and mercury lamps as calculated from the data of BARNES and FORSYTHE (**4, 5, 6, 8**). The lamps were on for 15 hours daily.

The largest and most vigorous plants of China aster, with the most profuse flowering, occurred with the incandescent radiant energy, with an intermediate growth and flowering response under the fluorescent. The plants under the mercury were small and averaged only one flower bud per plant.

TABLE I

GROWTH AND FLOWERING WITH EQUAL POWER CONSUMPTION

LAMP SOURCE*	PLANT MATERIAL									
	HEART OF FRANCE ASTER					NOBEL SPINACH				
	NO. PLANTS	AV. HT.	AV. DRY WT.	TOP-ROOT RATIO	AV. NO. FLOWER STALKS	NO. PLANTS	AV. HT.	AV. DRY WT.	TOP-ROOT RATIO	AV. NO. FLOWER STALKS
		cm.	*gm.*				*mm.*	*gm.*		
Incandescent 700 fc	12	46.9	8.1	9.6	9.5	20	5.2	1.4	14.4	0
Fluorescent, white 1200 fc	12	21.4	4.8	6.6	3.0	20	52.3	1.8	21.5	0.74
Mercury 1200 fc	12	18.6	3.8	7.7	1.0	20	14.7	0.6	25.0	0.18

* Same power consumption per square foot for all sources.

The spinach plants grown under the fluorescent were vigorous and dark green, and 74% had flower stalks. Those under the mercury were much less vigorous, with flower stalks on but 18% of the plants. With the incandescent lamp, spinach was small and had no flower stalks or even microscopic floral primordia as indicated by micro-dissection methods.

BALANCED RADIANT ENERGIES

COMPARISON OF UNFILTERED SOURCES.—Incandescent (1000-watt standard), mercury, and white and daylight fluorescent lamps (30-watt) were used over Nobel spinach, Biloxi soybean, and Indiana Baltimore tomato. The radiant energy was balanced for the various sources to approximately 800 footcandles directly under the lamps. The mercury and incandescent lamps were arranged in one plot so that there were varying proportions of mercury and incandescent radiant energy across the plot as indicated in the tables. A 15-hour photoperiod was used.

The tomato and soybean plants were seeded directly in the gravel and the

TABLE II

RESPONSE OF NOBEL SPINACH TO RADIANT ENERGY AT 800 FC FOR 15 HOURS FROM VARIOUS SOURCES WHEN GROWN AT 20° C. AND 25° C.

LAMP SOURCE	20° C.				25° C.		
	NO. PLANTS	AV. FRESH WT.	AV. DRY WT.	AV. NO. MATURED LEAVES	NO. PLANTS	AV. FRESH WT.	AV. DRY WT.
		gm.	*gm.*			*gm.*	*gm.*
Incandescent-mercury							
1*	26	5.1	0.37	9.8	15	1.8	0.14
6†	45	3.1	0.23	8.1	24	1.8	0.12
3‡	23	1.4	0.10	6.3	30	1.7	0.10
Fluorescent, white	....	....	.....	.....	24	1.7	0.13
Fluorescent, daylight	29	3.0	0.17	6.9	23	1.7	0.13

* 1: 7 to 25% Hg.
† 2: 25 to 86% Hg.
‡ 3: 86 to 95% Hg.

spinach in a sand-peat mixture. The spinach was transplanted to the appropriate compartments on germination. Air temperatures were controlled at 15°, 20°, and 25° C. within the limits previously designated.

The growth results are given in tables II, III, and IV. It should be noted that the growth at the various temperatures cannot be compared directly as the experiments were neither conducted simultaneously nor for the same length of time. Only the data within one temperature range can be compared.

TABLE III

RESPONSE OF BILOXI SOYBEAN TO RADIANT ENERGY AT 800 FC FOR 15 HOURS FROM VARIOUS SOURCES WHEN GROWN AT 20° C. AND 25° C.

LAMP SOURCE	20° C.			25° C.			
	NO. PLANTS	AV. FRESH WT.	AV. DRY WT.	NO. PLANTS	AV. HT.	AV. FRESH WT.	AV. DRY WT.
		gm.	*gm.*		*cm.*	*gm.*	*gm.*
Incandescent-mercury							
1*	20	6.6	0.80	27	57	8.1	1.0
2†	42	5.1	0.66	21	64	6.0	0.7
3‡	23	4.0	0.48	25	64	5.2	0.6
Fluorescent, white	....	.....	.....	20	48	7.0	0.7
Fluorescent, daylight	55	4.7	0.51	21	39	6.3	0.8

* 1: 7 to 25% Hg.
† 2: 25 to 86% Hg.
‡ 3: 86 to 95% Hg.

TABLE IV

RESPONSE OF INDIANA BALTIMORE TOMATO TO RADIANT ENERGY AT 800 FC FOR 15 HOURS FROM VARIOUS SOURCES WHEN GROWN AT DIFFERENT TEMPERATURES

LAMP SOURCE	15° C.§				20° C.§				25° C.§			
	NO. PLANTS	AV. HT.	AV. FRESH WT.	AV. DRY WT.	NO. PLANTS	AV. HT.	AV. FRESH WT.	AV. DRY WT.	NO. PLANTS	AV. HT.	AV. FRESH WT.	AV. DRY WT.
		cm.	*gm.*	*gm.*		*cm.*	*gm.*	*gm.*		*cm.*	*gm.*	*gm.*
Incandescent-mercury												
1*	28	36.0	21.8	1.33	18	29	6.9	0.39	30	27	5.0	0.28
2†	20	35.0	16.9	0.93	28	30	7.2	0.37	26	23	3.8	0.18
3‡	33	22.0	10.5	0.57	26	21	4.8	0.23	32	18	2.3	0.12
Fluorescent, white	19	9.3	5.8	0.37	.....	.....	.....	.....	22	10	1.8	0.11
Fluorescent, daylight	26	8.3	5.7	0.35	41	8	3.2	0.19	22	9	2.1	0.11

* 1: 7 to 25% Hg.
† 2: 25 to 86% Hg.
‡ 3: 86 to 95% Hg.
§ The plants in the different temperature treatments were not of the same age when harvested, and therefore cannot be directly compared with one another.

When mixtures of incandescent and mercury arc radiant energy were given the plants, there was a decrease in height and weight as the amount of mercury arc irradiation was increased. A desirable vigorous stocky growth, retaining the increased weight feature secured under the incandescent alone, was not obtained at any mixture of the two types of irradiation.

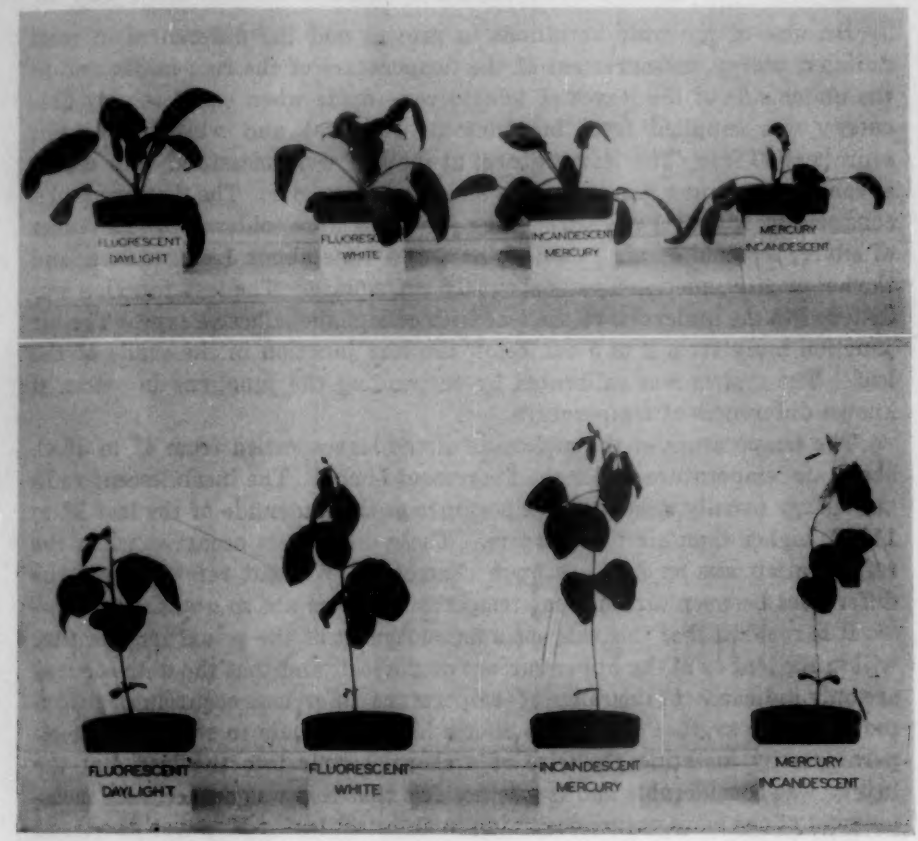

FIG. 1. Nobel spinach (above) and Biloxi soybean (below) at 20° C. under fluorescent and combinations of incandescent and mercury radiation. Plant second from right had 7–25% mercury and the plant on the right, 86–95% mercury. (Tables II and III.)

The greatest production of dry matter for all of the species occurred under the incandescent–low mercury combination. Here the growth was tall and spindling at 20° and 25° C. At 15° C., the plants were stocky and vigorous.

Very satisfactory growth was secured with the 800 footcandles of white and daylight fluorescent radiant energy with all of the species, except at 15°. The tomato plants were small, dark green and grew slowly at this temperature. The white fluorescent in some cases gave somewhat better growth as evidenced by increased weight and height than did the daylight, but the differences were very slight. At 15° and 20°, the tomato plants under the

fluorescent were short and stocky, with marked anthocyanin pigmentation, a blue-green color, and long petioles and short internodes. At 25°, the color was lighter green, there was little anthocyanin, which disappeared as the plants became older, and the internodes were longer than at 15° and 20°.

Temperature measurements

Because of the wide variations in growth and the differences in total radiated energy, measurement of the temperature of the root media and at the under side of the leaves of tomato were made when equal total visible energy was supplied from incandescent (500 fc) and white fluorescent sources (900 fc). The temperatures at the leaf were measured with a pair of constantin-copper thermocouples and a galvanometer. The thermocouples consisted of No. 40 AWG wire. The junctions were soldered to thin vanes of silver, the cold or air junctions having a vane about 1 cm. square, and the hot or leaf junction a vane about 0.5 cm. square. The leaf junction was fastened to the underside of the leaf with cellophane adhesive tape. The air junction hung from 2 to 5 cm. below the leaf junction in the shade of the leaf. The system was calibrated by suspending the junctions in water at known differences of temperature.

The temperature at the underside of the leaves varied from 4° to 5° C. above air temperature under the fluorescent lamps. The incandescent radiant energy usually raised the temperature at the underside of the leaf 9° to 11° C. higher than air temperature. These differences occurred when the refrigeration was on in the growth chambers. Without refrigeration, the differences between air and leaf temperatures were not so great.

It is realized that this was not a measurement of the actual temperature within the leaf or at the upper surface of the leaf, and that the data secured are only indicative of the range of temperature differences occurring between the two lamp sources. Previous efforts had been made to secure leaf temperatures by inserting the wire of a thermocouple into the leaf, but the injury was considerable and it was not felt that this was an accurate measurement of the temperature occurring in an intact leaf. However, the trend and magnitude of differences were similar to those secured by the method reported above.

The temperature of the root media at 5 cm. below the surface was from 3° to 6° C. higher directly under the incandescent lamps than under the fluorescent, the variation depending on the number of hours the lamps had been on previous to measurement and factors such as the length of time following irrigation.

Filtered mercury arc and incandescent

Radiant energy.—In view of the results secured with the mercury arc radiation showing the low efficiency of dry weight production, it was considered that possibly the strong near ultraviolet radiant energy at 3654 A and the high proportion of energy in the blue might be producing an in-

hibiting effect. In order to test this hypothesis, filters were prepared as dyed gelatin films poured on glass, which completely absorbed the 3654 A line in one case and the 3654 A line and the group of lines in the blue in the other.

The filters were prepared by mixing equal parts of dye and a gelatin stock solution, pouring the mixture on carefully levelled panes of glass, 64 cm. square, at the rate of 20 ml. of mixture (10 ml. stock gelatin and 10 ml. of dye solution) per 100 sq. cm. of glass surface. The stock gelatin solution

TABLE V

TRANSMISSION OF GELATIN FILTERS USED TO ABSORB SHORTER WAVELENGTHS OF THE MERCURY ARC SPECTRUM

WAVELENGTH	FILTER NO. 1 (ABSORBING 3654 A LINE) TRANSMISSION	FILTER NO. 2 (ABSORBING 3654, 4047 AND 4358 A LINES) TRANSMISSION
mμ	%	%
320	0	0
330	0	0
340	0	0
350	0	0
360	0	0
365	0	0
370	0.5	0
375	6.0	0
380	16.0	0
385	30.0	0
390	36.0	0
400	64.0	0
410	76.0	0
420	81.0	0
430	.....	0
440	.....	0.5
450	81.0	3.0
455	.....	7.0
460	.....	14.0
470	.....	34.0
480	.....	53.0
490	.....	68.0
500	88.0	77.0
520	.....	86.0

consisted of 150 gm. gelatin and 50 gm. sorbitol dissolved per liter of water. Filter No. 1, absorbing the 3564 A line, contained 25 mg. naphthol disulfonic acid and 25 mg. hydroxy naphthoic acid per 100 sq. cm. of glass surface. Filter No. 2, absorbing the 3654, 4047 and 4358 A lines, contained 25 mg. picrolonic acid per 100 sq. cm. glass surface. The transmission of the two filters are given in table V.

Four experimental conditions were set up at equal total energies, including (1) high pressure mercury arc with a film of undyed gelatin on glass interposed; (2) high pressure mercury arc filtered so that the 3654 A mercury line was absorbed; (3) high pressure mercury arc filtered so that the 3654, 4047 and 4358 A lines were absorbed; and (4) incandescent irradiance

TABLE VI

RESPONSE OF INDIANA BALTIMORE TOMATO TO THE REMOVAL OF THE SHORTER WAVELENGTHS OF THE MERCURY ARC SPECTRUM AND TO INCANDESCENT RADIANT ENERGY

TREATMENT*	AV. FRESH WT.	AV. DRY WT.	DRY MATTER	TOP-ROOT RATIO	AV. HT.
	gm.	*gm.*	%		*cm.*
Mercury arc, unfiltered	8.7	0.43	4.9	21	24
Mercury arc, 3654 A line removed	9.3	0.46	4.9	28	26
Mercury arc, 3654, 4047, and 4358 A lines removed	1.3	0.07	5.1	11	13
Incandescent	29.3	2.42	8.2	39	77

* Twenty-five plants per treatment.

of equal value in total visible radiant energy to (1). The irradiance was adjusted to an average of 270 fc for the mercury treatments and to 175 fc

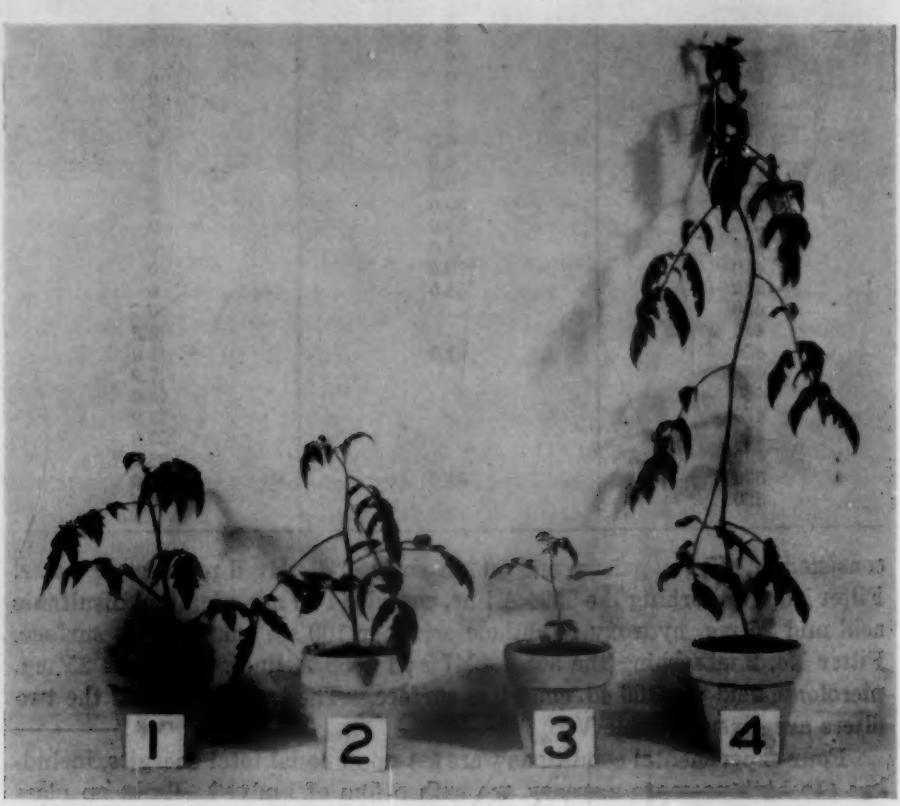

FIG. 2. Response of tomato to the removal of the shorter wavelengths of the mercury arc spectrum. (1) Full radiation from 400-watt H–1 mercury arc lamp. (2) Radiation from 400-watt H–1 mercury arc lamp, 3654 A line absorbed by filter. (3) Radiation from 400-watt H–1 mercury arc lamp, 3654, 4047 and 4358 A lines absorbed by filter. (4) Radiation of equal total visible energy from an incandescent lamp.

for the incandescent. A 15-hour photoperiod was employed. The mercury lamps were turned on 15 minutes prior to the incandescent so that they were at full irradiance at the beginning of the photoperiod. The temperature was maintained at 20° C. The treatments included 25 plants each, which were under the radiation variables from germination to harvest at the end of six weeks irradiation.

The results are given in table VI and figure 2. These data indicate that removal of the 3654 A mercury line had no significant effect on the growth of tomato. However, when the blue lines were removed, a very great reduction in growth and dry weight occurred. This is to be expected since the

TABLE VII

GROWTH AND FLOWERING RESPONSE OF NOBEL SPINACH TO VARIOUS SOURCES OF ARTIFICIAL RADIATION AT 20° C.

Radiation treatment	No. plants	Plants flowering	Av. ht.	Av. fresh weight		Av. dry weight		Dry matter
				Tops	Roots	Tops	Roots	
Series I. Equal footcandles. Radiation treatments begun 12 days from seeding for 55 days; 18-hr. day								
		%	cm.	gm.	gm.	gm.	gm.	%
Incandescent 750 fc	56	5	3.0	64.5	3.2	4.2	0.28	6.6
Incandescent, water filter 750 fc	46	80	9.0	40.8	2.3	3.0	0.19	7.4
Fluorescent* 750 fc	37	100	24.4	44.3	1.3	2.5	0.09	5.6
Series II. Equal total radiant energies in the visible. Radiation treatments begun 12 days from seeding for 42 days; 24-hr. day								
Incandescent 500 fc	45	89	26.8	28.1	1.2	2.3	0.10	8.0
Incandescent, water filter 500 fc	47	100	68.0	32.5	1.1	2.3	0.11	7.1
Fluorescent* 900 fc	48	100	31.3	24.8	0.8	1.9	0.07	7.6

* White.

blue lines of the mercury coincide with the blue maximum of the chlorophyll absorption curve. The yellow and green lines are in the region of minimum chlorophyll absorption, and therefore, even though there is a great deal of energy radiated in these lines, it is relatively ineffective in promoting carbon assimilation and the poor growth that resulted was undoubtedly due to this low photosynthetic rate. Under this condition, 25 per cent of the plants died before the 6 weeks of treatment were finished.

Comparison of water-filtered and unfiltered incandescent radiant energy with fluorescent radiant energy

It was thought that perhaps the tall weak growth secured with plants grown under unfiltered incandescent irradiation as compared with fluores-

cent might be partly due to the rise in temperature of the root media and to the differential increase in temperature of the leaves under the incandescent. It could be determined whether this were the case if the incandescent lamp were filtered with water so that the far infrared radiant energy were removed and no great differential increase in temperature of the leaves and the root media occurred.

TABLE VIII

GROWTH RESPONSES OF BILOXI SOYBEAN TO VARIOUS SOURCES OF ARTIFICIAL RADIATION AT 20° C.

RADIATION TREATMENT	NO. PLANTS	AV. HT.	AV. FRESH WEIGHT		AV. DRY WEIGHT		DRY MATTER
			TOPS	ROOTS	TOPS	ROOTS	
SERIES I. EQUAL FOOTCANDLES HARVESTED 24 DAYS AFTER SEEDING; 18-HR. DAY							
		cm.	*gm.*	*gm.*	*gm.*	*gm.*	%
Incandescent 750 fc	55	36.4	10.0	1.32	1.36	0.12	12.2
Incandescent, water filter 750 fc	48	54.1	6.8	1.23	0.85	0.08	11.5
Fluorescent† 750 fc	51	30.5	4.7	0.96	0.54	0.06	10.6
SERIES II. EQUAL TOTAL RADIANT ENERGIES IN THE VISIBLE HARVESTED 31 DAYS AFTER SEEDING; 18-HR. DAY							
Incandescent 500 fc	42	51.2	11.6	1.10	1.70	0.13	14.4
Incandescent, water filter 500 fc	40	74.2	8.4	0.80	1.06	0.10	12.7
Fluorescent† 900 fc	40	40.3	5.0	0.58	0.62	0.05	11.8
Greenhouse*	80	34.1	6.4	1.06	0.84	0.83	12.3
SERIES III. EQUAL TOTAL RADIANT ENERGIES IN THE VISIBLE HARVESTED 34 DAYS AFTER SEEDING; 24-HR. DAY							
Incandescent 500 fc	27	77.0	30.8	1.9	4.4	0.21	14.1
Incandescent, water filter 500 fc	32	87.4	18.5	1.6	2.6	0.12	13.5
Fluorescent† 900 fc	36	66.4	14.2	1.9	2.0	0.19	13.5

* Average 10-hour winter day.
† White.

Indiana Baltimore tomato, Nobel spinach and Biloxi soybean were grown under controlled temperature and incandescent (750-watt standard) and fluorescent radiation (30-watt white) conditions as previously described and indicated in the tables. For the water-filtered incandescent, the radiant energy was filtered through five inches of water. The results for the three species are given in tables VII, VIII, and IX.

TABLE IX

GROWTH RESPONSE OF INDIANA BALTIMORE TOMATO SEEDLINGS TO EQUAL TOTAL VISIBLE RADIANT ENERGIES FROM VARIOUS SOURCES OF ARTIFICIAL RADIATION AT 20° C.

RADIATION TREATMENT*	NO. PLANTS	AV. HT.	AV. FRESH WEIGHT		AV. DRY WEIGHT		DRY MATTER
			TOPS	ROOTS	TOPS	ROOTS	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	%
Incandescent ..... 500 fc	20	43.0	21.3	1.6	1.40	0.134	6.3
Incandescent, water filter ..... 500 fc	20	30.6	18.3	2.3	1.18	0.153	6.0
Fluorescent† ..... 900 fc	20	10.0	9.9	1.6	0.84	0.140	8.5

* Radiation treatments begun 10 days from seeding for 30 days; 15-hr. photoperiod.
† White.

The height and weight of the spinach plants were dependent largely on whether the flowering condition prevailed or not. When equivalent footcandles were used from the various sources at 750 fc for 18 hours daily, only 5% of the plants under the incandescent flowered, while 80% flowered under the water-filtered incandescent and 100% under the fluorescent. The flowering results with spinach under unfiltered incandescent may have been

FIG. 3. Nobel spinach at equal footcandles of white fluorescent, incandescent and water-filtered incandescent radiant energy at 20° C. (Table VII.)

due to the comparative temperature increase, although Nobel spinach has not been shown to be temperature sensitive in its qualitative flowering responses under greenhouse conditions (**21**). When equal total energies in the visible were used and the plants were given 24 hours at 500 fc incandescent (filtered and unfiltered), 89% of the spinach plants flowered in the unfiltered incandescent, and 100% under the filtered incandescent and the comparable fluorescent treatment (900 fc). Where fairly comparable flowering percentages

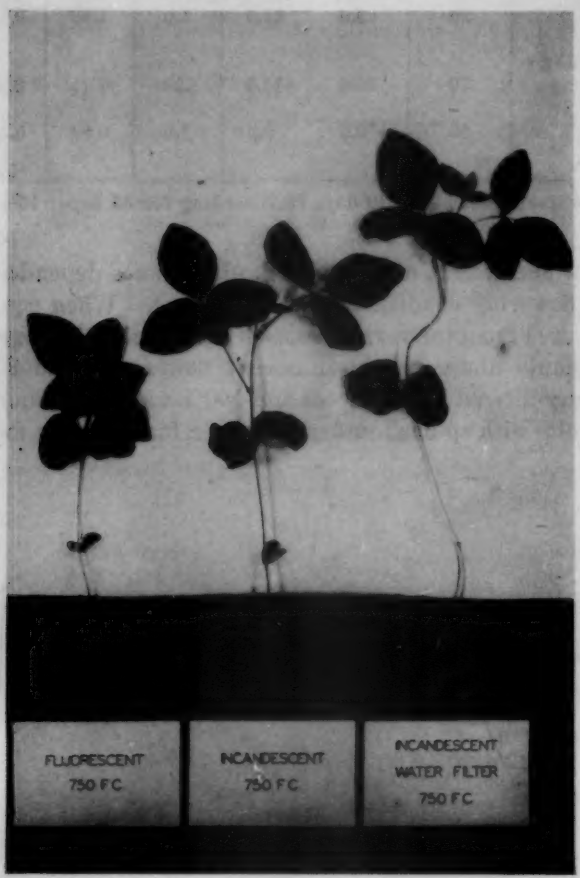

FIG. 4. Biloxi soybean at equal footcandles of white fluorescent, incandescent and water-filtered incandescent radiant energy at 20° C. (Table VIII.)

existed, the greatest height and fresh weight of the plants occurred when the incandescent was filtered with water. The dry weights were similar for the two incandescent treatments. As was found previously, the plants under the fluorescent were shorter and had less fresh and dry weight.

Biloxi soybean gave uniform trends in growth with the various sources regardless of irradiance or daylength. The total fresh and dry weights were, in order of decreasing magnitude: incandescent, filtered incandescent,

and fluorescent. The heights decreased from the filtered incandescent, through the incandescent to the fluorescent. Percentage dry matter was greater for the incandescent.

Tomato was decreased in height from the filtered incandescent as compared with the incandescent, with the shortest plants in the fluorescent. The fresh and dry weights of the tops decreased in the same order. The heavier roots were in the fluorescent and the percentage dry matter was least with the filtered incandescent.

FIG. 5. Indiana Baltimore tomato at equal total visible radiant energies from white fluorescent, incandescent and water-filtered incandescent sources at 20° C. (Table IX.)

Discussion of results

The results show that, of the three sources used in this investigation, the incandescent and fluorescent lamps are the only ones which produce reasonably satisfactory growth. The mercury arc is inefficient in supporting growth, probably due to the fact that the two most powerful lines in the visible spectrum occur in the green and yellow at 5461 and 5770–5791 A, respectively, where the chlorophylls have a minimum absorption. The blue lines at 4047 and 4358 A are responsible for only slightly more than one-third of the total visible radiant energy of the H–1 mercury arc and are the only lines which coincide with the chlorophyll absorption bands. These considerations appear to account in part for the low efficiency of the mercury arc. Removal of the strong 3654 A line in the near ultraviolet did not produce any significant effect, thus indicating that this line cannot be considered as producing any appreciable depressing effect on growth.

In general, the greatest dry weight production and height occurred with incandescent sources, whether compared on the basis of equal total electrical power consumption per plant, equivalent footcandles, or equal total visible energies. This indicates that the incandescent lamp is more efficient in the production of dry matter than either the daylight or white fluorescent lamps.

The principal disadvantage of the incandescent lamp is that it produces a plant with relatively long internodes, long petioles, and weak stems as compared with the growth obtained under solar irradiation. Data presented by POPP (**15**) and others, show that for high irradiances, blue radiant energy produces a shorter plant than red; under the longer wavelengths, the plant is tall, has long internodes, and is somewhat etiolated in appearance. However, supplementing the incandescent lamp with mercury arc irradiation did not appreciably improve the situation. Possibly supplementing the incandescent lamp with a blue-rich source as the blue fluorescent might prove useful in this connection, but this was not tried in these experiments.

A second disadvantage of the incandescent is that the radiated infrared causes the temperature of the leaves and root media to increase appreciably above the air temperature. There are several alternatives which can be used to partially correct this situation. The radiant energy can be filtered through water, which removes a little over one-third of the total radiated energy. Also the growth chambers can be operated at a reduced temperature to partially compensate for the higher leaf and media temperatures. Adequate circulation of air is necessary with high wattage unfiltered incandescent lamps. Where constant temperature conditions cannot be maintained, it is practically impossible to use unfiltered incandescent lamps to furnish the sole radiant energy for plants growing in small enclosed areas.

The temperature effects do not appear to be primarily responsible for the tall weak plants, elongated internodes, and long petioles secured with the incandescent. Removing the far infrared from the incandescent source with water so that the temperatures of the leaves and root media were not appreciably increased, as compared with fluorescent irradiation, appeared, in some cases, to increase the magnitude of the etiolated response rather than to decrease it. However, the temperature differences are responsible, in all probability, for some of the differences in synthesis of dry matter and for certain of the growth responses.

The plants were shorter, stockier and had darker green leaves with the fluorescent sources used than with the incandescent, and a superior growth form despite the fact that the total dry matter produced was considerably less than that produced under the incandescent. The fluorescent lamps have a somewhat larger proportion of the total energy emitted in the visible where it is useful to the plant than does the incandescent. A lesser proportion is in the infrared and much of this is dissipated by convection and conduction so that the lamps may be placed much closer to the plants than is possible with incandescent sources. With 30-watt fluorescent lamps, 3500° K. white, it is possible to secure 1200 footcandles directly beneath the lamps, if the lamps are mounted on a white reflecting surface at 1½-inch centers. A choice of fluorescent lamps is available so that a differential distribution of wavelengths is possible. However, none of the lamps can be used for critical spectral studies since their transmission bands are not sharp enough for most work of this type.

On the whole, an entirely satisfactory artificial source of radiant energy for plant growth is not yet available. However, the fluorescent sources appear to best meet requirements for general experimental work where radiant energy conditions are not to be a variable, despite the slower growth rates secured, since the plant form more nearly resembles that secured with daylight. Combinations of fluorescent lamps employing various phosphors may give improved results as compared to either the white or daylight, especially if a transmission curve similar to the chlorophyll absorption curve or the photosynthesis curve could be secured.

It should be pointed out as a consideration in working with artificial sources, that experimental data secured with any artificial source cannot be directly applied to interpretation of phenomena observed with solar radiant energy. Because no source has the same spectral distribution of energy as sunlight, it is impossible to predict that plant responses to such variables as photoperiod, irradiance, and others will be similar to those secured under greenhouse or field conditions.

Summary

Comparative growth responses of certain herbaceous annuals to several sources of artificial radiant energy were investigated. The sources included unfiltered incandescent, water filtered incandescent, high pressure mercury arc lamps, and white and daylight fluorescent lamps. The plant material irradiated was: China aster, variety Heart of France; spinach, variety Nobel; soybean, variety Biloxi; and tomato, variety Indiana Baltimore. The studies were made at 15°, 20°, and 25° C. and at varying photoperiods.

The greatest height and fresh and dry weight occurred with the incandescent sources. The water-filtered incandescent condition often produced taller plants with a lower percentage of dry weight than was the case with the unfiltered incandescent. The shortest plants, with the smallest fresh and dry weights, occurred with the high pressure mercury arc lamp. Removing the 3654 A line in the near ultraviolet spectrum of the mercury arc lamp failed to produce any significant increase in growth, indicating that this line is not responsible for the poor growth secured with this source. When the incandescent and mercury sources were set up in a single plot so that there were varying percentages of mercury and incandescent radiation, the height and weight decreased as the percentage of mercury radiant energy was increased. The plants under the fluorescent lamps were of an intermediate type of growth which was vigorous and stocky except at 15°. At 15°, the tomato plants were hard and grew slowly, a typical low temperature response. The white fluorescent gave a very slightly increased growth in some cases as compared with the daylight fluorescent lamp.

While there was greater production in fresh and dry weight under the incandescent lamp sources, the growth, on a whole, was tall and spindling except at 15° C. with tomato, and was not of a desirable type for general studies. Filtering the incandescent lamps through water to eliminate the

differential rise in temperature of the leaves and root media as compared with the fluorescent lamps failed to produce a stockier growth, but rather accentuated the etiolated type of response in some cases. It appears that the differences in growth responses secured with the various sources can be correlated principally with the differences in the respective spectral characteristics of the lamps.

The same trend in the results as summarized above were secured whether the energies were balanced on the basis of equal power consumption per square foot, equivalent footcandles, or equal total visible energies. The general trend was the same for 15-, 18-, and 24-hour photoperiods for the plant material used, except where differential flowering responses were secured.

It is concluded that no entirely satisfactory artificial sources of radiant energy for plant growth are available, but that the fluorescent sources appear to best meet requirements for general experimental work.

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THE THERMODYNAMICS OF ACTIVE (NON-OSMOTIC) WATER ABSORPTION

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In recent years, several workers (**1, 2, 12, 14, 15**) have brought forward evidence of a "secretion pressure" or "non-osmotic force" which is supposed to control the movement of water into (and out of) living plant tissues by causing an "active" (non-osmotic) uptake of water. So much of this evidence is indirect and controversial that papers expressing the opposite views have appeared (**6, 7, 17**). Since the postulated phenomenon depends on the expenditure of respiratory energy, it seems advisable to determine whether the conclusions are thermodynamically possible.

Perhaps the strongest argument in favor of active water absorption is the recognized fact of active solute absorption. It seems axiomatic that if a plant is capable of the latter, it should also be capable of the former. The concentration of K ions in the cell sap of Valonia has been shown to be 20 parts per thousand in excess of that outside the cell (**20**). If we assume a similar excess of a corresponding anion, this may mean a diffusion pressure of as high as 15–20 atms. However, Valonia cells have a volume of one to several cm.[3] If we assume that the cells of most higher plants are spherical with a radius of 5–50 μ (or non-spherical and of larger dimensions) the specific area $\left(\frac{\text{area}}{\text{volume}} \text{ or } \frac{1}{\text{radius}}\right)$ is of the order of 200 to 2000 times that of Valonia cells. Consequently, the cells of higher plants would have to expend 200 to 2000 times as much energy per unit volume as Valonia cells in order to maintain such a gradient.

The values obtained for higher plants are somewhat less [100 milliequivalents K per liter in excess of that in the surrounding solution (**9**)]; this is equivalent to a pressure of about 4½ atms. This gradient, however, is between the root cells and the surrounding soil solution. Consequently, the area separating the diffusion gradient is immensely smaller than the total cell area of a plant. In order to maintain a similar gradient between the protoplasm and vacuole of plant cells, it would require the expenditure of thousands of times as much energy as to maintain the above K gradient between roots and soil.

There is another factor, however, that must be considered. The energy required to maintain a negative gradient for any substance is proportional to the permeability of the cell to that substance; i.e., to the rate of diffusion of the substance across the membrane separating the two different concentrations. Since the cell is far more permeable to water than to any other substance normally occurring in it, far more energy would have to be expended to maintain a negative gradient for water than for any other substance.

The permeability constant for water is of the order of 10^{-7} M/cm.^{2}/sec. for a gradient of 1 M; that for K is of the order of 10^{-12} to 10^{-14} M/cm.^{2}/sec. for gradients of 0.01 to 0.06 M [(**4**) tables XIV and XXXI]. Therefore, at least 1000 times as much energy would have to be expended to maintain the same negative gradient for water as for potassium, which is one of the most rapidly penetrating ions.

Thus the energy used to maintain the high concentration of potassium in plant cells is sufficient to maintain a diffusion gradient for water of only the most minute fraction of one atmosphere.

If a negative diffusion gradient is to be maintained, some mechanism must be available to "pump" water against the gradient as rapidly as it is diffusing with the gradient. Consequently, work must be done equal to the product of the force × the distance moved:

$$W = Fs \tag{1}$$

Since by definition F = pa and s = vt

$$W = pavt \tag{2}$$

where p = the negative diffusion pressure against which water is moved; i.e., the "active" pressure.

a = the area across which the gradient occurs.

v = velocity of diffusion of water.

t = time.

In order to maintain cgs units, p (the pressure in atms.) must be converted to dynes/cm.^{2}; i.e., $\times 10^6$. If A is taken as specific area, the resulting quantity is the work done/cm.^{3} tissue or /gram tissue (approximately); v, the velocity of diffusion of water, can be obtained from the permeability of the cell to water. This value has been determined for several plant cells. When expressed in μ/hr./atm. at 20° C., it is 10–20 for Fucus eggs (**16**); 33 for Salvinia (**10**); and 21 for onion bulb cells (**11**). Since these values do not differ greatly from each other, a value of 20 will be used. Converting to cm., it becomes 20×10^{-4} cm./hr./atm. From this value, the velocity of diffusion v, becomes Pp where P = permeability and p = diffusion gradient in atms. Thus

$$W = 10^6 paPp = 10^6 p^2 AP \text{ ergs/hr./gm. tissue} \tag{3}$$

The only source of energy in the plant for such work is the respiratory process. Consequently, in order to determine whether any particular negative gradient is thermodynamically possible, it is necessary to calculate the amount of sugar that would have to be respired in order to do this work.

Taking 4.185 as the mechanical equivalent of heat and 3.75 kg. cal./gm. as the heat of combustion of glucose, the amount of sugar respired would be

$$S = \frac{10^6 p^2 AP}{10^7 \times 4.185 \times 3.75 \times 10^3} = 6.4 \times 10^{-6} p^2 AP \tag{4}$$

or, if P is taken as 2×10^{-3} cm./hr./atm.

$$S = 1.28 \times 10^{-5} p^2 A \tag{5}$$

This gives the gm. glucose/gm. tissue/hr. that would have to be respired in order to maintain the negative diffusion gradient of p atms.

It should be noted that this is a minimum value, assuming that all the respiratory energy released is utilized for this purpose; i.e., 100% efficiency. And it does not matter what the mechanism of active absorption is, this minimum amount of energy must be utilized.

Analysis of published results

BENNET-CLARK *et al.* (**1**) compared the plasmolytic and cryoscopic values for the osmotic pressures of six kinds of plants. In most cases the plasmolytic values were about 50% higher than the cryoscopic values, though some plants yielded no significant differences. They concluded that the difference is due to a "secretion pressure." According to their calculations, the "secretion pressure" amounted to 2.6–7.1 atms. in the case of the beets they tested. Thus p can be taken as having an average value of 5 atms. A, the specific area, is the tonoplast area/cm.^{3} Beet root cells are large, but the largest are not more than 0.5 mm. long and 0.2 mm. wide. They are not perfect cylinders, but if we use these maximum figures and assume them to be such, we shall certainly be underestimating the specific area. The cytoplasm layer is barely detectable in these cells, consequently cell area can be used for tonoplast area. From these values, specific area of beet cells (A) can be estimated.

$$A = \frac{2\pi rl + 2\pi r^2}{\pi r^2 l} = \frac{2(l + r)}{rl}.$$

where $l = .05$ cm.

$r = .01$ cm.

$A = 240$ cm.^{2}/cm.^{3}

If we now apply equation (5)

$$S = 7.7 \times 10^{-5} \text{ gm. glucose/gm. tissue/hr.}$$

Since the dry matter of a beet is about 15% of the fresh weight, this means that the beet would respire all of its dry matter in less than 3 months in order to maintain the negative diffusion gradient of 5 atms., even if all the respiratory energy were used for this one purpose.

In a later paper, BENNET-CLARK and BEXON (**3**) determine the respiratory rate of beet tissue. The highest values obtained by them were about 150 μl gas/hr./gm. fresh tissue. In terms of glucose utilized, this is equivalent to 2×10^{-4} gm. glucose/gm. tissue/hr. Consequently, even the maximum respiration rate of beet tissue, which occurs temporarily under conditions of stimulation, is only about 2.5 times the rate that would be required to maintain the negative diffusion gradient postulated by BENNET-CLARK *et al.*, if all the respiratory energy released were utilized for this sole purpose.

These calculations prove that their hypothesis of a "secretion pressure" cannot account for the differences they obtained between plasmolytic and cryoscopic values. The true explanation is therefore to be sought elsewhere.

Other workers, following up their results, have also found differences between the plasmolytic and cryoscopic values. ROBERTS and STYLES (**17**) obtained good agreement in the case of some conifer leaves but large differences with others. They attribute the differences to the presence of mucilaginous colloid. CURRIER (**6**) repeated the work with beets. In most cases the plasmolytic values were no more than 10% larger than the cryoscopic values, though in one series he obtained the same 50% difference found by BENNET-CLARK *et al.* His explanation is similar to that of ROBERTS and STYLES. He suggests that water squeezed out of the wall and protoplasm dilute the sap expressed from the frozen and thawed tissues. His own calculations, however, show that the protoplasm and wall do not contain enough water to account for the difference. Similarly, the colloid in the plants used by ROBERTS and STYLES cannot hold enough water to account for the differences of as much as 21.5 atms. that they obtained.

To check the experimental results of BENNET-CLARK *et al.*, the author determined the plasmolytic and cryoscopic values for red beets. A whole

TABLE I

CRYOSCOPIC AND PLASMOLYTIC O.P. VALUES FOR BEETS

BEETROOT	Δ JUICE	Δ ISOTONIC $CaCl_2$	$\frac{\Delta \text{ ISOTONIC } CaCl_2}{\Delta \text{ JUICE}}$
	°C.	°C.	
1	0.885	1.02	.....
2	0.82	1.02	.....
3	0.91	0.99	.....
4	0.885	1.02	.....
5	0.93	0.99	.....
6	0.89	1.02	
Total	5.32	6.06	1.14

beet was sliced, sections were cut from near the middle of the center slice for plasmolytic determinations, and the other slices were frozen for cryoscopic determinations.

In agreement with BENNET-CLARK *et al.*, the plasmolytic method yielded higher values than the cryoscopic (table I). But the increase is only 14% instead of the 50% obtained by them. Similar results were obtained with a set of beets having a freezing point lowering of about 1.5° C. It has long been known that such a difference exists, but this has usually been ascribed to the fact that the cryoscopic value is obtained with the normal cell sap, the plasmolytic value with sap concentrated by the exosmosis of water accompanying the loss of turgor on plasmolysis.

BENNET-CLARK *et al.* state that the ratio of the volumes of turgid : flaccid cell can be obtained from measurements of the linear dimensions of thin slices before and after plasmolysis. According to them, "in the case of the three beets used, the shrinkage was found to be between 2 and 4%." From this they conclude that the value of the ratio in the beet roots examined was between 1.00 and 1.05.

It is not clear from their statement whether the volume shrinkage or linear shrinkage is between 2 and 4%. If it is the latter, then the volume ratio will be between 1.06 and 1.12 instead of 1.00 and 1.05. This would agree better with results usually obtained for potato tuber tissue. Thus the two ratios calculated from LYON's (**12**) results (table I) are 1.04 and 1.13, respectively.

To check this, some measurements were made on beet roots (table II) taken from the same lot as those used for the determinations in table I.

The average linear ratio was 1.05, which agrees with the values given by BENNET-CLARK *et al.* for the volume ratio. The true value for the volume ratio is 1.16. Thus the plasmolytic value is 14% larger than the cryoscopic value, and the decrease in cell volume that occurs during the determination of the plasmolytic value is 16%. Consequently, in these beets the "secretion pressure" postulated by BENNET-CLARK *et al.* is non-existent.

From these results it is evident that part of the discrepancy between the plasmolytic and cryoscopic values of BENNET-CLARK *et al.* is due to their

TABLE II

DETERMINATIONS OF THE RATIO OF TURGID : FLACCID CELL IN BEETROOTS. EACH VALUE AN AVERAGE OF 6–10 STRIPS

ORIGINAL LENGTH OF TISSUE STRIP	LENGTH IN HYPERTONIC $CaCl_2$	LINEAR RATIO	VOLUME RATIO
mm.			
30.0	28.50	1.055	
29.0	27.75	1.045	
29.5	28.15	1.050	AV. 1.16

underestimation of the change in cell volume on loss of turgidity. CURRIER's values (2.2–7.3%) also seem too low, possibly due to the fact that one measurement was made on normal tissue, the other on infiltrated tissue. Changes in intercellular volume may conceivably mask part of the change in cell volume. Judging from cell measurements, however, the maximum cell shrinkage is not likely to be more than 20%, and therefore cannot wholly account for the differences obtained by BENNET-CLARK *et al.*

The method they used for plasmolytic determinations may also have been at fault. They deny the possibility of adhesion of protoplast to cell wall, partly because no wall deformation was seen. Such a deformation is not likely to be seen since it would be in the vertical plane of the section. The method used by the author avoids such an error, since the cells are always strongly plasmolyzed first, then allowed to deplasmolyze in successively weaker solutions until incipient plasmolysis is detected. This method has the added advantage of producing convex plasmolysis even in cells with the most viscous protoplasm. Traces of convex plasmolysis are much more readily detected than traces of concave plasmolysis. The use of $CaCl_2$ as plasmolyte avoids changes in concentration such as occur in sugar solutions due to the activity of microorganisms. The more rapid diffusion of $CaCl_2$ permits equilibrium to be reached much sooner than with sugar solutions.

BENNET-CLARK *et al.* do not mention whether or not they determined the freezing point lowerings of the sugar solutions used. If the osmotic pressures were simply calculated from the concentration, the plasmolytic values are almost certain to be high.

The wide spread in their plasmolytic values for different cells of the same root may indicate some factor such as adhesion or simply too great a variability between cells to obtain an average for the whole tissue from the 100 cells examined. The author always found it possible to distinguish three solutions: a hypertonic solution producing slight plasmolysis in all the cells; an isotonic solution producing incipient plasmolysis in about half the cells; and a hypotonic solution producing plasmolysis in few or none of the cells. The isotonic solution usually differed from the other two by about $\frac{1}{2}$ atm.

There are several other possible explanations. The smaller samples used by them to determine the freezing point lowering of the juice may have introduced larger errors because of the condensation of moisture on the cold tissues or due to inability to express a large enough fraction of the juice from such small samples to be representative (the first fraction would be more dilute). Thus their results varied from 0.67 to 0.83° C. for six different cylinders obtained from the same beet. The variations in our results for six different beets were only half as great.

Thus there are many sources of error, the sum total of which might conceivably lead to large differences such as were obtained by BENNET-CLARK *et al.* That these differences are not real is indicated by thermodynamic calculations, by our failure to obtain them, and by the great variability in the results obtained by BENNET-CLARK *et al.*

MASON and PHILLIS (**14**) assumed that the juice expressed from living leaves was the vacuolar sap and that remaining in the leaves was protoplasmic. On the basis of this assumption, they found that the O.P. of the "vacuolar sap" of cotton leaves was 2.4 atms., that of the "protoplasm" 19.3 atms. They concluded that the difference represented a "secretion pressure."

MASON and PHILLIS used the blades of cotton leaves freed of large veins. The cells are mainly cylindrical and not more than 100 μ long by 20 μ in diameter. From these dimensions, the specific area (A) is 2200 cm.^{2}/gm. Consequently from equation (5)

$$S = 8.1 \times 10^{-3} \text{ gm. glucose/gm. tissue/hr.}$$

From MASON and PHILLIS' own measurements of the respiration rate, it can be calculated that 0.088 gm. dextrose were respired/100 gm. tissue/hr.; i.e., the actual rate of respiration is about $\frac{1}{10}$ as rapid as would be required to maintain the negative diffusion gradient even if all the respiratory energy released were used for this sole purpose.

The fantastically high amount of energy that would have to be expended by the protoplasm in maintaining this negative diffusion gradient makes it quite clear that MASON and PHILLIS' "vacuolar" and "protoplasmic" fractions are in no way related to the true vacuolar and protoplasmic components in the living cell.

MASON and PHILLIS postulated that the pressure produced protoplasmic "fissures" which allowed the passage of unaltered vacuolar sap through the protoplasm. They give no reason for this hypothesis other than that it fitted in with their other assumption that the sap is unaltered by passage through the living cells. The fact that the concentration of the juice remained constant as the pressure was increased indicates that no such fissures developed, since they would enlarge with the increased pressure. Furthermore, since it required pressures of 400 atms. (6000 lb./in.^{2}) to obtain the expressed juice, and since the living cells plasmolyzed in a solution with an O.P. of 22.8 atms., it is inconceivable that these pressures should fail to force water through the unaltered plasma membrane. Consequently, their "vacuolar sap" was really almost pure water which they had filtered through the plasma membrane.

Using two methods of calculation (both based on the assumption that the liquid expressed from living tissues represents vacuolar sap) they conclude that the cell consists of 70% protoplasm and 30% vacuole.

CHIBNALL (**5**) gives analytical values for spinach leaves from which these fractions can be calculated. The total protein N varied from 2.9–3.8% of the dry weight during a period in which the dry weight varied from 16–11% of the fresh weight. On a fresh weight basis, this gives a value of 0.45% N. But only $\frac{1}{7}$ of this was cytoplasmic N, the rest being chloroplast N. And since the cytoplasmic protein had an N content of 16.25%, the amount of cytoplasmic protein was therefore 1.385 gm./100 gm. fresh tissue. From analyses of the protoplasm of many organisms, it has been found that it contains 7–10% protein (**19**). This gives a value of 19.8–13.85 gm. cytoplasm/100 gm. fresh tissue. The cytoplasm of spinach leaves therefore cannot occupy more than $\frac{1}{5}$–$\frac{1}{7}$ of the cell volume. The true value is likely to be even lower than this since, in view of the high O.P. of leaf cells, the cytoplasm of leaves is undoubtedly less highly hydrated than that of the slime moulds and animals on which the 7–10% value is based.

Whether or not the same values would be obtained with cotton leaves, we cannot say. MASKELL and MASON (**13**) give N values of 1% of the fresh weight for cotton leaves, which is double the value used above. However, this represents total N, which must be considerably higher than protein N. The values for cotton leaves are therefore probably of the same order as those for spinach.

Observational methods also show quite clearly that their calculated results are in error. They claim to have observed that the protoplasm occupies more of the cell than does the vacuole. To check on their observations, the author vitally stained sections of cotton leaves with neutral red. As in most leaves, practically the whole protoplast stained, the cytoplasm forming an almost invisible layer around the stained vacuole.

Every experimental value obtained by them gives further evidence of their misinterpretation. Thus the expressed juice had a pH of 7.0 to 7.2, the residue 5.7. Since protoplasm is known to have a pH about 7.0 (**8**) and

vacuolar sap is always acid [usually below pH 6 (18)] the above fractions cannot be vacuolar sap and protoplasm respectively.

BENNET-CLARK *et al.* (2) used MASON and PHILLIS' method to express juice from leaves containing colored vacuolar sap. The presence or absence of color in the expressed juice enabled them to judge whether or not they obtained vacuolar sap. When the pressures were applied gradually, the expressed juice was colorless. This juice was therefore almost pure water, filtered through the semi-permeable protoplasm layers. When, however, the leaves were subjected to sudden large increments of pressure, the expressed juice was strongly colored and had considerably higher osmotic values (7.86 atms. for *Fagus sylvatica* when 156 atms. pressure was suddenly applied; 3.75 atms. when 174 atms. pressure was applied gradually). Juice expressed from the killed residue (frozen in dry ice) was less intensely colored in the case of *Fagus sylvatica* and had higher osmotic values. BENNET-CLARK *et al.* conclude that MASON and PHILLIS' interpretation is correct when pressures are applied suddenly; i.e., the juice from live tissue represents vacuolar sap and that from the killed residue cytoplasmic sap. They also accept MASON and PHILLIS' concept of protoplasmic "fissures," through which the sap supposedly passes unaltered.

On the basis of these assumptions, they obtained differences of 6–19 atms. between the osmotic pressures of the "cytoplasmic sap" and "vacuolar sap." These results are of the same order as those of MASON and PHILLIS; consequently, the amount of energy required to maintain the negative diffusion gradient is again fantastically high.

The reasons for their interpretation are not given. It is obvious that if water can be filtered through the protoplasm when pressures are gradually applied (which BENNET-CLARK *et al.* proved), it will also be filtered through the protoplasm if those same pressures are suddenly applied. If the sudden application of pressure should produce protoplasmic "fissures" small enough not to injure the cells, then the expressed juice would be a mixture of unaltered or relatively little altered sap and filtered sap. The greater the pressure applied, presumably the greater the proportion passing through the fissures. Increased pressures would then result in increased osmotic pressures which is precisely what BENNET-CLARK *et al.* obtained. The mere fact that successive fractions *always* gave higher O.P. values is proof that they did not obtain unaltered vacuolar sap. The juice expressed from killed residue must therefore have a higher O.P. than that pressed from the live tissue. This result is in no way connected with the O.P. of the vacuole and cytoplasm respectively.

Since they never expressed all the juice (total amounts from 28 to 72% of the fresh weight were obtained) it is obvious that the average O.P. of all the expressed fractions would have to be considerably lower than that of the normal cell. Here again, this is simply due to the partial filtration of the juice and there is no need to seek any other explanation of the fact that plasmolytic values were higher than these averages.

It is interesting to note that of the six species tested by them, only one (*Fagus sylvatica*) gave higher concentrations of anthocyanin in the juice expressed from the living leaves than from the killed residue. In spite of this, BENNET-CLARK *et al.* conclude that the former represents vacuolar sap, the latter protoplasmic sap.

The above calculations prove that due to the enormous specific surface of the cell and its very high permeability to water, the living cell is unable to maintain a diffusion gradient of several atmospheres between the protoplasmic water and vacuolar water. In the case of the beet tissue used by BENNET-CLARK *et al.*, a gradient of 1 atmosphere would require fully 1/60 of the total maximum respiratory energy released under temporary stimulation or 1/30 when respiration is at the normal rate. In the case of the cotton leaves used by MASON and PHILLIS, 1/30 of the total respiratory energy released would be just sufficient to maintain this same 1 atmosphere gradient. Since so many other energy-consuming processes are simultaneously taking place in the cell, it is doubtful if this large fraction could be so used, and we can conclude that the energy available is certainly incapable of maintaining a diffusion gradient of more than 1 atm. between the cell sap and protoplasm. Since, however, the energy needed is directly proportional to the square of the gradient (equation 4) enough would be available to maintain a gradient of a fraction of an atmosphere. Whether the mechanism for this process exists in the cell is another question.

LYON (**12**) determined the "non-osmotic force" from the difference between the "calculated net osmotic pressure" and the "observed net osmotic pressure." Aside from the impossibility of obtaining a force by subtracting one pressure from another, it would be less confusing if we compared the two calculated values of wall pressure, for it is really these that he is comparing. We will avoid the use of LYON's symbols.

One value is calculated on the assumption that the wall pressure equals the osmotic pressure (O) minus the "net osmotic pressure"; i.e., the suction pressure (S) or diffusion pressure deficit. We will call this W_1.

$$W_1 = O - S \tag{6}$$

The other is calculated on the assumption (not stated by LYON) that Hooke's law holds for the cell wall; i.e., wall pressure is proportional to wall extension (and therefore approximately to cell volume). We will call this W_2.

Let us assume with LYON that there is a "non-osmotic force" producing an "active" uptake of water. We will use the symbol A for the pressure produced by this "active" uptake. Then

$$W_3 = O + A - S \tag{7}$$

On the basis of LYON's hypothesis, W_1 would be the incorrect value, W_2 and W_3 the correct ones. Therefore $W_2 = W_3$.

But $W_3 > W_1$ (by an amount equal to A).

Therefore $W_2 > W_1$.

Actually LYON finds that $W_1 > W_2$; e.g., in table I $W_1 = 10.35$ and

$W_2 = 5.77$. It follows that he has no evidence whatsoever for a "non-osmotic force" unless it has a negative value; i.e., the water would be pumped out of the cell instead of absorbed!

This does not, of course, explain why he did obtain such large differences between W_1 and W_2. If we consider the assumption on which W_2 is based, it can be seen that this is valid only if the wall is not stretched beyond its elastic limit, for then Hooke's law does not hold.

To determine whether or not this is true, the author tested LYON's technique. Strips of potato tissue ($20 \times 1 \times 1$ mm.) were allowed to shrink in isotonic dextrose and measured from time to time. At the end of one hour (which was found to be sufficient for equilibrium to be reached), the strips were transferred to tap water. They were measured at the end of four hours (the time used by LYON) and then returned to the isotonic dextrose. Equilibrium was again reached at the end of one hour, but to make sure, the final measurements were made after 18 hours. These checked with the ones at the end of 1 hour. The results are shown in table III.

TABLE III

LENGTH OF STRIPS OF POTATO TUBER TISSUE (AVERAGE OF 6)

ISOTONIC DEXTROSE (0.60 M)	WATER	2ND IMMERSION IN ISOTONIC DEXTROSE
mm.	*mm.*	*mm.*
20.5	24.5	22.0

Thus the elastic stretch at the end of 4 hours in tap water was only 62.5% of the total stretch. LYON's value for W_2 is 56% of the value obtained from the classical equation $W = O - S$, or 58% if he had calculated wall pressure from the increase in length of the strip instead of the increase in volume (Hooke's law refers to a linear stretch). Now if the total stretch were multiplied by 0.625 to eliminate the fraction due to plastic stretch, there would be no significant difference between the two values for W. The two percentages agree well enough, considering that they were obtained with different tubers.

It is obvious, therefore, that wall pressure cannot be calculated on the assumption that Hooke's law holds, unless a correction is made for plastic stretch (permanent set). If, however, the cell wall of the normally turgid potato tuber cell is not stretched beyond its elastic limit, a correction such as made above should be valid.

If such a correction is made, it should be possible to determine the true value for A in equation (7). But does this quantity A really represent the postulated pressure due to "active" absorption of water? It must be remembered that the quantity O represents not the osmotic value of the cell sap but of the living protoplast, since it was determined by the method of minimum cell volume. Consequently, any pressure due to active absorption of water

is included in this quantity. Any further work along the lines attempted by LYON will therefore fail to shed light on the problem of "active" water absorption.

In contrast to the above investigators, VAN OVERBEEK (**15**) has produced strong evidence to show that the root pressure of a growing tomato plant is partly due to non-osmotic forces. He obtained small negative diffusion gradients (1–1½ atms.) between the osmotic pressure of the xylem exudate and the suction pressure of the roots surrounding it. These differences were obliterated by KCN. The plants used were growing actively and therefore were in a high state of metabolic activity. The area across which the gradient was maintained was simply the area of the stele or endodermal layer. The specific surface is $\frac{2r_s}{r_r^2}$ where r_s = stele radius and r_r = root radius. Assuming the ratio $\frac{\text{stele radius}}{\text{root radius}}$ has a value of about ⅓, the specific surface is therefore 0.22 cm.²/cm.³ root tissue. This is only 1/1000–1/10,000 that of the tonoplast specific surface calculated above.

From equation (5), we can calculate that the amount of sugar that would have to be respired in order to maintain the negative gradient in VAN OVERBEEK's experiments is 2.82×10^{-9} gm./gm. tissue/hr.

It would therefore require the energy released by the respiration of approximately 0.3 micrograms sugar/100 gm. tissue/hr. to maintain this negative diffusion gradient. Thus from the point of view of energy requirement, VAN OVERBEEK's results are well within the realm of possibility.

Summary

1. A simple formula is derived for calculating the energy needed to maintain a negative diffusion gradient in plant tissues.

2. Calculations reveal that respiratory energy is incapable of maintaining a negative gradient of more than 1 atmosphere between the osmotic pressures of the vacuole and cytoplasm of a cell.

3. A critical examination of the higher values obtained by other workers reveals that these values are due to incorrect interpretations.

4. In absorbing roots, a negative gradient of 1 atm. between the stele and the tissues external to the stele could be maintained by the energy released from the respiration of 0.3 micrograms sugar/100 gm. tissue/hr.

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EFFECT OF ALTERNATING CONDITIONS OF BORON NUTRITION UPON GROWTH AND BORON CONTENT OF GRAPE VINES IN SAND CULTURE[1]

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(WITH THREE FIGURES)

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The effect of boron deficiency upon the growth and fruiting of the grape under field conditions has previously been reported by the author (**9, 10**). The field investigations led to further studies under controlled conditions; thus the present paper is concerned with the development of symptoms of boron deficiency and the accumulation and translocation of boron in the vine under alternating levels of boron nutrition of grape vines in sand culture.

Methods

Two-year-old vines of the varieties Catawba, Herbert, Golden Muscat, and Lindley were planted in 2.5-gallon glazed crocks filled with twenty-mesh washed white quartz sand and provided with a drainage hole. On March 23, 1943, twelve vines of each variety, carefully selected as to uniformity of size, were planted one to a crock. The vines were pruned back to two canes, and four buds were permitted to develop shoots on each vine. The crocks were arranged on a ground plot in the greenhouse in four varietal blocks of twelve vines each.

Two nutrient treatments, hereafter designated as "plus-boron" and "minus-boron," were used. The plus-boron solution contained: 170 p.p.m. of mono-potassium phosphate; 590 p.p.m. of calcium nitrate; 148 p.p.m. of magnesium sulfate; 5 p.p.m. of iron citrate; 0.91 p.p.m. of manganese chloride; 0.11 p.p.m. of zinc sulfate; 0.04 p.p.m. of copper sulfate; 0.045 p.p.m. of molybdic acid; and 0.5 p.p.m. of boron from boric acid. The minus-boron solution was identical in composition except for the omission of boron. The pH of the solutions was adjusted to approximately 6.0 by the addition of sodium hydroxide. The nutrient solutions were applied by flushing each crock with 500 ml. of the solution three times a week. On other days the plants were given distilled water.

The plus-boron and minus-boron treatments were applied to alternate vines in each varietal block. Seven weeks after planting, three of the plus-boron Golden Muscat vines were changed to minus-boron treatment and three minus-boron vines were changed to plus-boron treatment. A week later a similar change was made with the Catawba and Herbert vines. The nutrient-sand culture seemed well adapted for grapes since the plus-boron vines exhibited vigorous, normal foliage and each vine made a total shoot growth

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of ten to fifteen feet by the end of three months which is comparable to good field growth in the first season after planting.

Estimation of boron was made by the quinalizarin method (**6**). Since determinations of boron content of the vines under field conditions (**10**) had shown rather extreme differences existing in various parts of the vine; four classes of material were sampled for analysis: (a) lower leaves, consisting of mature leaves toward the base of the cane usually including the second to fourth leaves; (b) upper leaves, consisting of the four or five young, smaller leaves at the end of the shoot; (c) lower stem, the portion subtending the lower leaves; and (d) upper stem, that portion subtending the upper leaves. The upper leaves of class (b) were in actively growing condition in all normal plus-boron vines but were much older and apparently had ceased growth in the boron-deficient vines. All leaf samples included the petiole. Material from the three vines constituting a nutrient treatment were composited to form a sample. All samples were taken at the conclusion of the experiment (three months after planting) with the exception of a series taken from the Catawba and Herbert vines at the time of changing from plus to minus and from minus to plus boron nutrition.

Results

Deficiency symptoms

The first evidence of symptoms of boron deficiency were observed about one month after growth commenced. At this time the plus-boron Catawba vines had made a total shoot growth of about 120 cm., the Golden Muscat vines 70 cm., the Herbert vines 110 cm., and the Lindley vines 155 cm. The symptoms in the early stages were exhibited as (a) a diffuse yellowing or chlorosis of the younger leaves; (b) brownish, water-soaked areas developing in the apical tendrils; and (c) cupping of the third or fourth leaf from the shoot tip. The exact order of appearance of these symptoms varied among the varieties. The Golden Muscat vines first showed chlorosis while with the Catawba and Herbert vines, the water-soaked areas on the young tendrils appeared first. Later stages of the deficiency consisted largely of a progressive worsening of the earlier symptoms shown on the leaves and tendrils. The leaves became more cupped and rugose, the chlorosis developed between the veins, and, in extreme cases, entire areas of the leaf became necrotic. The tendrils developed transverse cracks and later died back from the tips. Death of the terminal bud, a well-recognized symptom of boron deficiency with many plants, did not occur until the leaf and tendril symptoms were quite advanced; finally, however, the terminal bud died and abscissed.

The apical internodes of the deficient vines were much shorter than those of the normal vines. All of the affected parts exhibited abnormal rigidity and brittleness. Young leaves appearing after the onset of the deficiency were often misshapen and malformed, and deeply-lobed with a cut-leaf effect. Deficiency symptoms did not develop on the lower leaves of the vine, even though the terminal parts were extremely affected. On rapidly growing

shoots of the grape, the fifth or sixth leaf from the tip was the oldest leaf to show boron deficiency and, on these, symptoms were usually confined to marginal chlorosis only. This is illustrated in figure 1, which shows the entire growth produced by a minus-boron Lindley vine. The three lower leaves showed no evidence of deficiency symptoms even though the extreme symptoms at the tip had developed a month before the photograph was taken.

FIG. 1. A Lindley grape vine grown under minus-boron nutrition in sand culture. Note that the lower leaves show no evidence of deficiency even though growth had ceased and terminal symptoms were severe a month before the photograph was taken.

Apparently the symptoms are developed only in those parts which are in active meristematic condition at the time of occurrence of the deficiency.

EFFECT OF ALTERNATING NUTRIENT SOLUTIONS

The vines which were changed from plus-boron to minus-boron culture and those changed from minus-boron to plus-boron will be referred to respectively as plus-minus and minus-plus boron vines. The minus-boron Golden Muscat and Catawba vines showed extreme symptoms at the time of change. Ten days after application of boron these vines gave evidence of recovery and in fifteen to twenty days the new growth was developing normally. Since the terminal bud had abscissed on these varieties, several lateral buds were forced, giving a bushy appearance to the canes when growth was resumed. A similar condition has been noted in the vineyard (9) and termed "witch's broom." The Herbert vines showed only the first stages of deficiency at the time of changing from minus to plus boron culture. The terminal buds of these varieties resumed growth. In all instances leaves of the minus-plus vines which showed deficiency at the time of changing to the plus-

boron solution retained all of those symptoms even though the new leaves were normal in growth and appearance. The occurrence of deficiency symptoms on the older leaves of a vine therefore would not necessarily indicate a boron deficiency existent in the vine at the time of observation.

The plus-minus boron vines were much slower in developing evidence of deficiency than the minus-plus vines were in recovering from the deficiency. The plus-minus Golden Muscat vines showed tendril necrosis twenty-five days after the change of treatment. A week later the symptoms had progressed to an advanced stage. The Catawba and Herbert plus-minus vines were even slower in exhibiting evidence of the deficiency, the first symptoms appearing in thirty days.

Growth of vines

Weekly measurement of the canes of each vine throughout the course of the experiment gave an opportunity to correlate linear growth with development of boron deficiency symptoms in the several treatments and varieties. Since a deficiency of boron affected primarily the apical meristematic areas, it is quite evident that the deficient vines should show a distinct curtailment of linear growth. The linear growth of the vines and the weight of the growth produced are given in table I. These data show the much greater growth of the vines in the plus-boron culture, and the effect on the total growth caused by the alternating conditions of boron nutrition. The shoot growth and weight of the minus-plus vines indicate clearly the recovery of the vines after boron was added to the culture solution. Conversely, the growth of the plus-minus vines shows the depressing effect of the omission of boron from the culture solution during the last month of the test.

Since it is of importance to recognize the earliest exhibition of a nutrient deficiency rather than the extreme symptoms, it is of interest to know if the growth rate of the canes of the vines was affected prior to the appearance of definite observable symptoms. Such a condition was observed (data not presented) in the case of the Golden Muscat and Lindley vines, in which there was a distinctly lower growth rate for one or two weeks before any foliar symptoms were apparent.

Boron content of the vines

The boron content, expressed in parts per million on a dry weight basis, of all parts of all varieties receiving boron in the nutrient solution was much higher than that of comparable parts of vines not receiving boron (table II). This difference was especially pronounced in the concentration of boron in the leaves, with the plus-boron leaves having three to six times as much boron as the minus-boron leaves. The plus-boron vines showed a much greater concentration of boron in the leaves than in the subtending stems. In the minus-boron vines the differences in boron content between the leaves and stems was insignificant, indicating an equalization of concentration in the parts of the boron deficient plants. The boron content of the plus-boron vines ranged from 56.8 to 146.0 p.p.m. in the leaves and from 29.0 to 41.4

TABLE I

LENGTH AND WEIGHT OF SHOOT GROWTH PRODUCED BY TWO-YEAR-OLD GRAPE VINES DURING THREE MONTHS' SAND CULTURE IN THE GREENHOUSE

Treatment	Herbert			Catawba			Golden Muscat			Lindley		
	Shoot length	Green wt.	Dry wt.	Shoot length	Green wt.	Dry wt.	Shoot length	Green wt.	Dry wt.	Shoot length	Green wt.	Dry wt.
	cm.	*gm.*	*gm.*	*cm.*	*gm.*	*gm.*	*cm.*	*gm.*	*gm.*	*cm.*	*gm.*	*gm.*
Continuous plus-boron, March 23–June 23	383	126.4	31.1	371	157.9	37.9	324	128.9	33.2	424	135.4	35.3
Continuous minus-boron, March 23–June 23	201	85.1	24.0	171	73.5	22.3	125	68.8	14.3	140	46.8	11.0
Plus-boron to May 21,* minus-boron theerafter	266	109.0	25.5	325	116.1	28.8	241	113.4	28.5	.....	.....	.....
Minus-boron to May 21,* plus-boron thereafter	393	120.8	26.0	246	108.5	24.4	218	107.4	19.8	.....	.....	.....

* Golden Muscat vines were changed in treatment on May 13.

p.p.m. in the stems. The boron content of the minus-boron plants ranged from 10.8 to 27.0 p.p.m. in the leaves and from 20.4 to 23.9 p.p.m. in the stems. It should be pointed out that the lower and upper leaves of the minus-boron Catawba and Herbert vines had a similar boron concentration, although the lower leaves were entirely normal in appearance while the upper leaves had exhibited advanced stages of boron deficiency for a prolonged period previous to sampling.

The boron content of those vines in which there was an alternation of boron nutrition is also given in table II. The withdrawal of boron from the culture solution resulted in greatly decreased boron content of both upper and lower leaves, when compared to the continuous plus-boron culture. Here

TABLE II

THE BORON CONTENT OF GRAPE VINES AFTER THREE MONTHS' GROWTH IN GREENHOUSE SAND CULTURE

TREATMENT	PORTION OF VINE ANALYZED	BORON ON OVEN-DRY WT. BASIS			
		CATAWBA	HERBERT	GOLDEN MUSCAT	LINDLEY
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Continuous plus-boron, March 23–June 23	Lower leaves	77.4	119.6	123.3	87.8
	Upper leaves	93.3	146.0	56.8	109.2
	Lower stem	29.3	29.0	.....	.....
	Upper stem	41.1	41.4	.....	.....
Continuous minus-boron, March 23–June 23	Lower leaves	27.0	24.4	12.7	21.6
	Upper leaves	23.4	23.6	15.8	10.8
	Lower stem	22.6	20.4	.....	.....
	Upper stem	21.6	23.9	.....	.....
Plus-boron until May 21, minus-boron thereafter*	Lower leaves	35.8	35.8	42.2	.....
	Upper leaves	26.3	31.7	16.9	.....
	Lower stem	39.0	25.0	.....	.....
	Upper stem	44.1	25.2	.....	.....
Minus-boron until May 21, plus-boron thereafter*	Lower leaves	152.4	90.9	46.3	.....
	Upper leaves	60.6	105.3	45.3	.....
	Lower stem	41.1	28.3	.....	.....
	Upper stem	65.8	47.3	.....	.....

* Golden Muscat vines changed in treatment May 13.

again, there is apparently a tendency toward equalization of boron concentration in the various plant parts under conditions of a low boron nutrition level. With addition of boron to the culture solution, the boron level of the minus-plus vines closely approached or even exceeded that of the continuous plus-boron vines at the end of the test. It will be noted that the lower leaves and stems of the minus-plus vines had a boron content greatly in excess of that in the continuous minus-boron vines, indicating a build-up of boron in these tissues after the inception of the plus-boron nutrition.

Thus far, only comparative boron levels of the several treatments at the end of the experiment have been presented. Since with Catawba and Herbert, canes were removed for sampling at the time of change of treatment from plus to minus boron nutrition and vice versa, there is opportunity to

compare the boron content of these plants at the time of change and a month later at which time the effects of the change of treatment were apparent in the foliar development. The differences in boron level of the leaves and stems brought about by the alternation of boron nutrition is shown graphi-

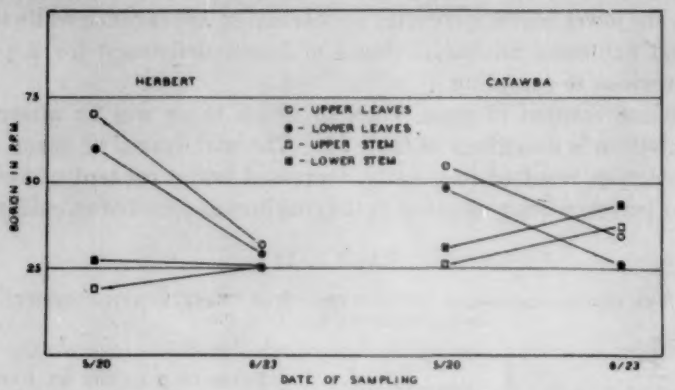

FIG. 2. Effect of changing from plus-boron to minus-boron nutrition upon the boron content of shoots of the grape. Treatments changed May 20th.

cally in figures 2 and 3. In general the previous data are substantiated. The change from a plus-boron to a minus-boron nutrient culture resulted in a much lower boron content of both lower and upper leaves although the stems showed slight change in boron level. The addition of boron to the

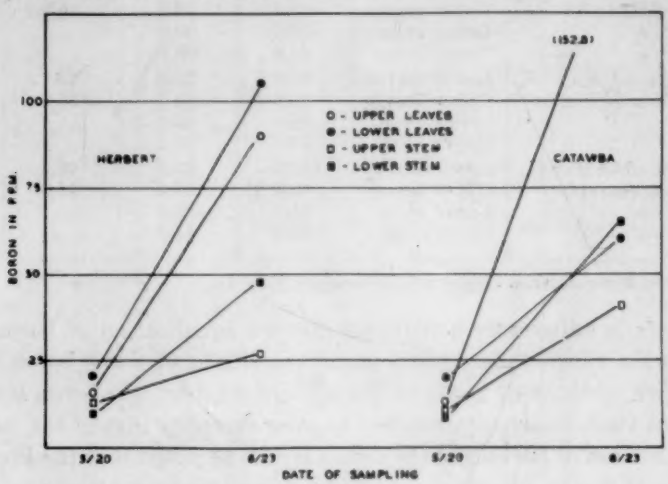

FIG. 3. Effect of changing from minus-boron to plus-boron nutrition upon the boron content of shoots of the grape. Treatments changed May 20th.

nutrient solution caused a great increase in the boron level of the vines grown previously in a minus-boron culture, with the highest level attained in the leaves.

Discussion

The data on growth measurement and boron content of the grape vines grown in the sand culture experiments contribute information on two problems of boron nutrition of plants concerning which there is found controversial or inconclusive statements in the literature. These problems involve: (a) the relationship between boron content of the plant and deficiency symptoms; and (b) consideration of the mobility or retranslocation of boron within the plant.

It has been found that plants exhibit luxury consumption of boron in the presence of an adequate supply, building up a content far above any critical level (**8**). Also BATJER (**1**) has found that the difference in boron content between normal and boron-deficient apple fruits may be very slight. Such findings and other factors present difficulties in an attempt to establish a definite relationship between the boron content of the plant and apparent deficiency symptoms. It is clear that in a boron-deficient grape vine the basal leaves, which are normal to all outward appearances, may be just as low or lower in boron than the upper leaves which may show extreme symptoms. Likewise, leaves with extreme boron symptoms may have a boron content equal to that of normal leaves if samples are analyzed following a period of adequate boron nutrition. Such apparently contradictory results are logical if it is assumed that only those leaves which are in actively meristematic condition can develop deficiency symptoms; that boron deficiency symptoms in mature leaves does not disappear when boron nutrition is resumed, although the leaves may increase greatly in boron content.

The above findings are pertinent to the problem of sampling and analysis of plants for the purpose of diagnosing boron deficiencies in the field. The boron content of mature leaves showing deficiency symptoms may in no wise indicate a below-minimum boron level, if the plant had had access to an adequate supply of boron just previous to sampling. The seasonal nature of the occurrence of boron-deficiency symptoms under vineyard conditions shown by the author (**10**) presents a situation regarding boron nutrition of the vine not dissimilar to that established in the alternation of boron nutrition in sand cultures. Thus there were found in the vineyard, shoots of the current season's growth exhibiting symptoms of boron deficiency on the basal and terminal leaves but with normal leaves in the medial portion.

The definite tendency for the vines to exhibit equalization of boron concentration under low-boron nutrition may possibly serve as a diagnostic index in the attempts to correlate deficiency symptoms with boron levels in the plant.

There seems to be a rather general agreement in the literature concerning the immobility of boron within the plant tissue and the subsequent need of a continuous supply furnished by the nutrient substrate for the maintenance of growth (**3, 7, 13, 15**). SHIVE (**11**) concluded that plants of tobacco and cotton cannot build up an available reserve of boron in their tissues which will sustain normal development during a later deficiency of this element.

However, SHIVE in a later paper (12); gives evidence of a portion of the boron within the plant existing in a mobile state by finding 28.7% of the boron in *Vicia faba* and 78.4% of the boron in the corn plant in the expressed sap of the tissues.

Certain of the results in the present experiment can be used as evidence concerning the mobility and transfer of boron in the current season's growth of the grape vine. The growth habit of the vine makes the grape peculiarly adaptable for such studies. In so far as the initial growth of the vines in the minus-boron treatment is concerned, it is impossible to definitely ascertain whether this growth utilized reserve boron in the roots and stem of the two-year-old vine or whether some boron was available in the sand culture media. However, it seems unlikely that the sand, after being thoroughly washed and leached at the beginning of the experiment, would provide available boron for the heavy initial growth which occurred for about thirty days before symptoms of deficiency were observed. Other workers have noted similar results. WEINBERGER and CULLINAN (14) working with one-year-old peach trees cut back to a single bud, obtained normal growth for a period of about six weeks in a minus-boron sand culture. JOHNSTON and FISHER (5) found that tomato plants upon removal to a minus-boron nutrient water culture, continued in growth for a period of about three weeks. It must be assumed that this growth necessitated the utilization of boron present in tissues laid down before the change in nutrient conditions was effected.

The relatively delayed appearance of deficiency symptoms after changing from a plus-boron to a minus-boron nutrition in the present experiment also suggests that there was utilization and transfer of reserve boron. It is interesting, and perhaps suggestive, that the period from the time of withholding boron from the nutrient solution until the appearance of deficiency symptoms, closely approximated the period from growth inception after planting until the initial symptoms of deficiency. Furthermore, the actual amount of cane growth made by the vines during these two periods was not greatly different.

More definite evidence of boron transfer within the vine is afforded by the analyses of the different parts of the vines of the several treatments. In all instances there was a definite reduction in boron content of the lower leaves on the canes of vines which were changed from plus-boron to minus-boron nutrition. Since these leaves were mature and inactive in growth at the time of change of treatment, their greatly lowered boron level could be explained only by assuming a transfer of boron to the upper and active meristematic regions. Since the stems of the plus-boron vines were as high in boron as the stems of the continuous boron vines, it follows that the leaves served largely for storage of reserve boron. This also would be assumed from the fact that the leaves of the vines built up a much higher content of boron under plus-boron nutrition than did the stems.

The apparent equalization of the boron level in various parts of boron-deficient vines of the plus-minus series may also be accounted for on the basis of transfer, since it was shown that a differential existed in these plants

before the deficiency developed. Although there is clear evidence that transfer of boron does occur, and that therefore it can be assumed that the boron in the grape vine is in a mobile state, it is likewise clear that transfer from the lower leaves can take place only when the boron content in these leaves is above a certain level. Boron present in the leaves at this critical level, which was about the same for the three varieties, presumably may be considered to exist in an immobile state insofar as transfer, adequate to maintain normal growth at the growing point, is concerned. Calculated on the basis of analyses of the continuous plus-boron vines, about two-thirds of the total boron in the lower leaves of Herbert and Catawba, and about half of that in Catawba, was transferred during the thirty-day period of minus-boron nutrition in the plus-minus treatment. Of course, the percentage of the total boron present in the mobile state would be greatly dependent upon the degree of "luxury" consumption and reserve accumulation of boron that has taken place.

Study of boron transfer within the plant by analysis of the plant parts was also made by BRANDENBURG (**2**) with the sugar beet. He found that when the boron supply was interrupted, the new leaves of the sugar beet were very low in boron content; the older leaves, formed while the boron supply was plentiful, retained the normal amount of boron. This result is in direct opposition to the present findings with the grape, in which the older leaves lose boron as the younger leaves are developed under minus-boron nutrition. It is conceivable, as suggested by EATON and BLAIR (**4**), that the organic form of boron in the tissue of different species of plants may be greatly different, resulting in a widely varying percentage of the boron present in soluble or mobile form.

From the data presented herein on the analyses of the plant parts and on the growth of the vines in the several treatments, it seems logical to conclude that there was a definite transfer of boron from the lower mature leaves on the cane of the grape upward to the terminal actively growing parts, and that the amount thus transferred was sufficient to maintain normal growth of the cane for a limited period, or until the lower leaves were depleted of boron to a certain level. With regard to the findings of other workers on the question of mobility of boron within the plant, it would seem more accurate to interpret their results on a quantitative basis. It is undoubtedly true that a plant cannot store sufficient boron to maintain normal growth for an indefinite period, but this is equally true of other plant nutrients. The conclusion of JOHNSTON and FISHER (**5**) to the effect that the boron in the plant cannot be used over again and, therefore, must not be considered in the nature of a catalytic agent in plant growth, must be accepted. Their conclusion, however, that boron is fixed in an immobile state in the plant definitely does not hold true for the grape.

Summary

1. Grape vines grown under minus-boron sand culture nutrition in the greenhouse, developed boron deficiency symptoms thirty days after inception

of growth. These symptoms were similar to those previously found under field conditions.

2. Boron-deficient vines resumed normal growth within ten days after changing to plus-boron nutrition. Normal vines changed to minus-boron nutrition continued to grow normally for 25 to 30 days before symptoms of the deficiency were apparent.

3. Only those leaves that were in a meristematic condition at the time of development of the deficiency showed deficiency symptoms. The older basal leaves on boron deficient vines remained normal in appearance even though the terminal leaves exhibited extreme symptoms.

4. In plus-boron vines the boron content of the leaves ranged from 57 to 146 p.p.m. and that of the stems from 29 to 41 p.p.m. In minus-boron vines the concentration of boron was more or less equalized in all parts of the vines at levels of approximately 20 to 25 p.p.m.

5. Vines changed from plus-boron to minus-boron nutrition showed definite lowering of the boron content in the lower mature leaves on the shoot to a point approaching the boron level of continuous minus-boron vines. Coincident with the decrease in boron in the lower leaves was the continued growth in length of the shoots for about 30 days after change of nutrition.

6. A discussion is presented concerning the problem of mobility and retranslocation of boron within plant tissues.

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SOME EFFECTS OF POTASSIUM DEFICIENCY ON THE NITROGEN METABOLISM AND OIL SYNTHESIS IN THE TUNG TREE (*ALEURITES FORDII*)

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It is noteworthy that the great amount of study devoted to the element potassium has revealed but little definite information as to its specific function in plants. One of the difficulties in assigning a definite role to potassium in plant metabolism is the fact that potassium is not known to occur in any definite organic compounds except salts of organic acids and proteins.

MILLER (**4**) has reviewed the work on the physiological role of potassium in plants, showing that the literature contains many conflicting reports. There appears to be no agreement on the function of potassium in either carbohydrate or protein metabolism. Some investigators, particularly the English workers (**3, 9**), have emphasized the importance of potassium in the formation of carbohydrates while others (**6, 13**) have reported that a deficiency of potassium results in an accumulation of carbohydrates. NIGHTINGALE (**5**) and WALL (**13**) have summarized the various investigations on the effect of potassium on nitrogen metabolism and, on the basis of their own work as well as that of others, concluded that potassium plays a prominent part in protein metabolism although adequate evidence of its specific function in such processes has not yet been obtained.

Potassium deficiency of the tung tree (*Aleurites fordii*), widespread in northern Florida and southern Georgia, is one of the major nutritional problems of that important tung-growing area (**1**). Preliminary field trials in a 4-year-old orchard have shown that the application of potassium fertilizers corrected the symptoms of interveinal chlorosis and necrosis associated with potassium deficiency (**7**). These plots provided an opportunity for investigating the effects of potassium deficiency on the metabolism of the tree, particularly with regard to oil formation. The effects of a supplementary nitrogen treatment were also investigated.

Materials and methods

In August 1941, 4-year-old trees were selected in a potassium-deficient area near Capps, Florida, and grouped on the basis of the severity of potassium-deficiency symptoms so that comparable plots were obtained for each treatment (**7**). Each of the following treatments was applied in the spring of 1942 to each of six single-tree plots and was supplementary to a blanket

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application of 4 pounds per tree of a 3–6–7 mixed fertilizer: (1) none, (2) five pounds of potassium chloride, (3) four pounds of sodium nitrate, and (4) three pounds of potassium nitrate. On each of three sampling dates, two composite leaf samples were taken from the trees of each treatment, one from replications 1, 2, and 3, and another from replications 4, 5, and 6. In compositing the leaf samples, 25 midshoot leaves were taken from each tree, thus making a total of 75 leaves per sample. Analogous composite fruit samples comprising five fruits from each tree were taken in the same manner and at the same time. Both leaf and fruit samples were obtained on June 26,[2] August 13, and October 21 to correspond with the respective periods (I) just preceding synthesis of oil in the kernels, (II) midpoint of active oil formation, and (III) maturation of the kernel, as indicated by previous studies (11).

The composite samples of leaves and of fruit were prepared and the analyses made essentially as described by SELL *et al.* (11) with the modifications introduced by GILBERT, SELL, and DROSDOFF (2). In the latter paper the methods are given for determining the various nitrogen fractions. The following determinations were made on both the leaf and fruit samples: dry matter, reducing sugar, non-reducing sugar, starch, acid-hydrolyzable polysaccharides other than starch, potassium, and total insoluble amide, and amino nitrogen. As the insoluble nitrogen is largely protein nitrogen, this term will be used for convenience in referring to the insoluble nitrogen fraction. In addition, oil in the kernels and the percentage of kernels in the whole fruit were determined.

Results

LEAVES

Data on leaf composition are given (table I). In all tables each value represents the average of the analyses of two composite samples. Since the original determinations were made in duplicate, the statistical significance of the mean differences between treatments and of the interaction of treatments with the sampling date was determined. Although these data were also calculated on a per plant-part basis, the data thus obtained showed no important trends other than those indicated by the percentage data. Consequently, the data on the per plant-part basis are not presented here.

First it should be noted that the potassium content was considerably higher throughout the season in the leaves of the potassium-treated trees than in those not treated with potassium. These differences had high statistical significance. Also the nitrogen treatment resulted in a highly significant increase in nitrogen content. This shows that fertilizer treatments were effective and that the concomitant changes in the organic reserves of the plant are associated with increased potassium and nitrogen due to treatment.

Leaf samples collected in June from the potassium-deficient trees showed

[2] Trees treated with 4 pounds of sodium nitrate were not sampled on June 26.

TABLE I

COMPOSITION OF LEAVES FROM UNTREATED AND POTASSIUM- AND NITROGEN-TREATED 4-YEAR-OLD TUNG TREES IN A POTASSIUM-DEFICIENT AREA NEAR CAPPS, FLORIDA, 1942

Sampling date	Treatment†	Constituents*									
		Dry matter	Reducing sugar	Non-reducing sugar	Starch	Polysaccharides other than starch	Total N	Protein N	Amide N	Amino N	K
		%	%	%	%	%	%	%	%	%	%
June 26	Check	46.8	4.50	4.36	4.70	4.42	1.92	1.80	0.03	0.08	0.67
	K	41.4	5.44	3.50	2.86	3.80	2.20	2.08	0.03	0.09	1.12
	KN	44.6	4.88	5.28	2.25	3.46	2.60	2.48	0.04	0.09	0.95
Aug. 13	Check	50.8	4.71	4.88	1.74	5.36	1.84	1.72	0.04	0.08	0.56
	K	46.1	4.98	4.12	3.34	5.44	1.86	1.74	0.03	0.08	0.85
	N	52.7	4.20	6.45	1.31	5.50	2.41	2.28	0.04	0.10	0.48
	KN	45.9	5.60	5.46	2.42	5.84	2.40	2.26	0.04	0.12	0.87
Oct. 21	Check	53.0	4.24	3.66	0.42	4.12	1.83	1.68	0.03	0.12	0.54
	K	48.4	4.72	3.84	0.68	3.70	1.88	1.71	0.03	0.13	0.91
	N	52.3	3.45	4.18	0.54	4.27	2.23	2.04	0.03	0.16	0.46
	KN	50.0	5.00	4.98	0.54	4.12	2.18	1.98	0.04	0.18	0.84

* Average of two composite samples, moisture-free basis.

† All trees received 4 lbs. per tree of a 3–6–7 fertilizer in March supplemented by the following treatments: Check = no additional fertilizer; K = 5 lbs. of KCl; N = 4 lbs. of $NaNO_3$; KN = 3 lbs. of KNO_3.

more starch and polysaccharides than those from potassium-fertilized trees. No visible deficiency symptoms were apparent at that time. By the August sampling date, however, the starch in the check trees had decreased markedly, reaching a level even lower than the earlier level of the potassium-treated trees which meanwhile had increased in starch (though this increase was not statistically significant). The higher starch content of the potash-treated trees in August had high statistical significance. At this time the abnormal leaf pattern due to potash deficiency was evident in both the check and nitrogen-only treated trees. This coincides with the period when both translocation of materials from the leaves to the fruit and oil formation are proceeding at a rapid rate. It is noted also that the nitrogen treatments effected a highly significant increase in non-reducing sugar at the August and October sampling dates.

Most of the nitrogen in the leaves is present in protein form, the amide and amino fractions being present only in small amounts. Tests for ammonium and nitrate nitrogen were negative. Therefore the nitrogen treatment primarily had increased the protein content of the leaves, although the increase in amino nitrogen attained high statistical significance. The potassium treatment is associated with an increased protein content of the leaves over the check at the time of the first sampling, but not in the later periods.

Fruit and its component parts

Data are presented on the analysis of the kernels (table II), and on the analysis of the hulls (table III). These data together with that on the analysis of the shells were combined to give the composition of the fruit as a whole (table IV). Shells were included with the kernel fraction at the first sampling period because of difficulty of mechanical separation at that stage. At other periods the shells were removed from the kernels and analyzed separately. Since composition of the shells was found to be quite similar to that of the hulls, data for this fraction are not presented except as included in the calculation of the composition of the whole fruit.

At the time of the first sampling in the latter part of June there was a highly significant increase in the percentage of potassium in the fruit, particularly in the hulls, due to potassium treatment. The nitrogen treatments increased the nitrogen content of the fruit, but to a lesser extent. Nitrogen treatments also resulted in a highly significant increase in the protein content of both kernels and hulls and in the amino nitrogen content of the hulls. At this date the polysaccharides in the kernels from fruit of potassium-plus-nitrogen-treated trees were higher than those from trees receiving other treatments. However, the polysaccharide content of the hulls was highest in fruit from untreated trees. No significant differences due to treatment were found in the other carbohydrate fractions.

At the time of the August sampling, oil formation was well under way and the effect of the potassium treatments on increasing the oil content of the kernels and the whole fruit was apparent. The increased percentage of

TABLE II

COMPOSITION OF KERNELS FROM UNTREATED AND POTASSIUM- AND NITROGEN-TREATED 4-YEAR-OLD TUNG TREES IN A POTASSIUM-DEFICIENT AREA NEAR CAPPS, FLORIDA, 1942

Sampling date	Treatment†	Constituents*										
		Dry matter	Reducing sugar	Non-reducing sugar	Starch	Polysaccharides other than starch	Total N	Protein N	Amide N	Amino N	K	Oil
		%	%	%	%	%	%	%	%	%	%	%
June 26	Check	9.98	3.68	6.66	2.18	7.43	1.61	1.07	0.19	0.36	1.18	.....
	K	9.60	3.74	6.14	1.88	7.96	1.68	1.12	0.20	0.37	1.30	.....
	KN	10.70	2.80	4.88	1.46	10.57	1.82	1.18	0.23	0.40	1.52	.....
Aug. 13	Check	22.32	Trace	2.72	Trace	4.28	3.66	2.27	0.24	1.15	0.86	30.9
	K	21.42	Trace	4.62	Trace	4.37	3.50	2.06	0.28	1.16	0.88	32.7
	N	23.20	Trace	2.56	Trace	4.28	3.79	2.27	0.26	1.26	0.68	28.6
	KN	30.18	Trace	2.22	Trace	3.78	3.72	2.40	0.28	1.04	0.86	33.3
Oct. 21	Check	96.40	0.77	11.31	Trace	8.88	2.22	1.75	Trace	0.48	0.62	62.3
	K	98.32	0.72	8.76	Trace	8.42	2.11	1.60	Trace	0.50	0.54	65.4
	N	98.51	0.76	5.94	Trace	6.80	2.86	2.24	Trace	0.62	0.63	62.4
	KN	98.55	0.23	5.72	Trace	7.12	2.75	2.32	Trace	0.44	0.58	65.4

* Average of two composite samples, moisture-free basis.

† All trees received 4 lbs. per tree of a 3–6–7 fertilizer in March supplemented by the following treatments: Check = no additional fertilizer; K = 5 lbs. of KCl N = 4 lbs. of $NaNO_3$; KN = 3 lbs. of KNO_3.

TABLE III

COMPOSITION OF HULLS FROM UNTREATED AND POTASSIUM- AND NITROGEN-TREATED 4-YEAR-OLD TUNG TREES IN A POTASSIUM-DEFICIENT AREA NEAR CAPPS, FLORIDA, 1942

Sampling date	Treatment†	Constituents*									
		Dry matter	Reducing sugar	Non-reducing sugar	Starch	Polysaccharides other than starch	Total N	Protein N	Amide N	Amino N	K
		%	%	%	%	%	%	%	%	%	%
June 26	Check	24.10	3.54	4.20	0.60	11.63	0.83	0.74	0.04	0.05	0.70
	K	21.92	4.80	4.13	0.74	9.47	0.88	0.76	0.02	0.10	1.27
	KN	22.90	3.66	3.28	0.89	9.24	1.14	0.95	0.05	0.13	1.65
Aug. 13	Check	25.14	2.90	3.54	0.36	8.17	0.59	0.48	0.03	0.08	1.08
	K	22.92	2.98	3.63	0.27	6.74	0.52	0.44	0.02	0.07	1.84
	N	25.76	3.79	3.68	0.29	6.95	0.90	0.68	0.06	0.15	0.95
	KN	24.48	3.48	3.39	0.26	7.14	0.78	0.62	0.03	0.13	1.80
Oct. 21	Check	77.8	0.32	Trace	0.66	9.09	0.31	0.31	Trace	Trace	1.96
	K	83.2	0.68	Trace	0.51	8.13	0.27	0.27	Trace	Trace	2.67
	N	83.0	0.80	Trace	0.58	8.80	0.44	0.44	Trace	Trace	1.45
	KN	84.4	0.38	Trace	0.52	7.30	0.42	0.42	Trace	Trace	3.08

* Average of two composite samples, moisture-free basis.

† All trees received 4 lbs. per tree of a 3–6–7 fertilizer in March supplemented by the following treatments: Check = no additional fertilizer; K = 5 lbs. of KCl; N = 4 lbs. of $NaNO_3$; KN = 3 lbs. of KNO_3.

TABLE IV

COMPOSITION OF WHOLE TUNG FRUIT FROM UNTREATED AND POTASSIUM- AND NITROGEN-TREATED 4-YEAR-OLD TUNG TREES IN A POTASSIUM-DEFICIENT AREA NEAR CAPPS, FLORIDA, 1942

Sampling date	Treatment†	Constituents*											
		Dry matter	Reducing sugar	Non-reducing sugar	Starch	Polysaccharides other than starch	Total N	Protein N	Amide N	Amino N	K	Kernels	Oil
		%	%	%	%	%	%	%	%	%	%	%	%
June 26	Check	19.10	3.56	4.64	0.90	10.86	0.97	0.80	0.07	0.11	0.79	18.4	.....
	K	17.72	4.60	4.50	0.95	9.20	1.02	0.82	0.06	0.14	1.28	18.5	.....
	KN	18.53	3.48	3.60	1.01	9.51	1.28	1.00	0.08	0.20	1.62	20.7	.....
Aug. 13	Check	27.97	2.18	2.95	0.26	8.06	0.84	0.62	0.05	0.18	0.88	9.2	2.88
	K	26.55	2.04	2.94	0.18	7.04	0.80	0.58	0.05	0.17	1.34	10.2	3.42
	N	28.92	2.72	2.95	0.20	7.33	1.11	0.78	0.08	0.25	0.76	10.2	2.96
	KN	29.34	2.32	2.56	0.16	7.22	1.14	0.82	0.07	0.25	1.32	14.7	4.90
Oct. 21	Check	84.10	0.42	3.52	0.38	8.79	0.89	0.75	Trace	0.14	1.12	30.2	18.84
	K	87.96	0.60	2.92	0.26	8.25	0.90	0.72	Trace	0.17	1.31	34.4	22.50
	N	87.78	0.64	2.00	0.31	8.13	1.26	1.05	Trace	0.21	0.76	34.1	21.29
	KN	88.82	0.28	2.10	0.27	7.54	1.30	1.14	Trace	0.16	1.44	37.6	24.62

* Average of two composite samples, moisture-free basis.

† All trees received 4 lbs. per tree of 3–6–7 fertilizer in March supplemented by the following treatments: Check = no additional fertilizer; K = 5 lbs. of KCl; N = 4 lbs. of $NaNO_3$; KN = 3 lbs. of KNO_3.

oil in the whole fruit resulted from an increase in the percentage of both oil in the kernel and of kernel in the fruit. The increased potassium content of the hulls (and consequently of the whole fruit) resulting from potassium treatment, was even more marked at this stage than at the first sampling. Carbohydrate fractions at this time showed no significant differences due to treatment. As at the time of the first sampling, protein content of the fruit was higher in the nitrogen-treated trees, and this increase had high statistical significance. Other nitrogen fractions showed no consistent differences due to treatment.

The effect of potassium treatments in increasing the oil content of the fruit was pronounced at the last sampling date with fully mature fruit. The increase in the percentage of oil in the kernels and in the percentage of kernels in the whole fruit from trees receiving the potassium treatments was highly significant statistically. The nitrogen treatments resulted in a highly significant increase in the protein content of both the kernels and the hulls and in the amino-nitrogen content of the hulls. Kernels from the untreated trees were high in non-reducing sugar which had not been converted to oil or protein. The potassium treatment without nitrogen lowered the sucrose content and increased the oil in the kernels but not the protein, in comparison with the check treatment. The nitrogen-alone treatment, on the other hand, did not significantly affect the oil content of the kernels but increased the amount of protein present, thus accounting for the lower sucrose content in comparison with the kernels from the check treatment. The potassium-plus-nitrogen treatment produced kernels with high oil, high protein, and low sucrose content.

The potassium treatments markedly increased the potassium content of the hulls but not of the kernels. Actually there was a trend toward a lower potassium content in the kernels as a result of potassium treatment, but this decrease did not attain statistical significance.

Discussion

The pronounced effect of potassium deficiency on the composition of tung fruit is the most striking observation in this investigation. The effect was clearly evidenced by the middle of August when oil formation was well under way. At that time the potassium-treated trees showed a higher percentage of oil in the kernel and a higher percentage of kernel than did the check trees. No significant differences in carbohydrate content were associated with any treatments at this stage. In the mature fruit, however, the kernels from the check trees showed an accumulation of sucrose, and this was associated with a lower oil content compared to that of the potassium-treated trees.

Since it has been shown that sucrose (**10**) is the most important carbohydrate reserve utilized in oil formation in mature tung kernels (**11**), it is logical to assume that this constituent may be considered as the storage form alternate to the oil and protein in the seed, accumulating when formation

of the other two reserves is checked. This suggests that potassium may function in the process of conversion of sugar into oil. The nitrogen-alone treatment has also resulted in a lower sucrose content than the check treatment, but this was associated with an increased amount of protein, the oil content remaining about the same. Other more extensive experiments with nitrogen fertilization of tung trees (**8, 12**) have shown that there is actually a decrease in the percentage of oil in the kernel with increased nitrogen fertilization, resulting in kernels with relatively high protein content.

In addition to effecting an increase in the oil content of the kernel, the potassium treatment has also resulted in an increased percentage of kernel in the whole fruit in comparison with the check trees, thereby substantially increasing the oil content of the whole fruit. These data are in agreement with the findings of other investigators (**8**) and the results of a number of other experiments being conducted at the tung laboratories of the Bureau of Plant Industry, Soils, and Agricultural Engineering of the U. S. Department of Agriculture.

With reference to the data on the leaves it would appear that potassium deficiency shows two stages. The early stage (June sampling), at which no symptoms are apparent, is characterized by an accumulation of starch and polysaccharides in the leaves. At the late stage (August sampling), starch tends to disappear from the leaves of potassium-deficient trees to a greater extent than from the leaves of the potassium-treated trees. At this time there is a rapid translocation of materials from leaves to fruit. This physiological pattern of potassium effects is similar to that found in other plants (**5, 14**).

Summary

During the growing season the composition of leaves and fruit of 4-year-old tung trees low in nitrogen and potassium was compared with that of trees fertilized with these two elements. From these data the following conclusions have been drawn:

1. The leaf data indicate that potassium-deficient trees show two stages; early in the season starch and polysaccharides accumulate, while later in the season they tend to disappear to a greater extent than from potassium-treated trees.

2. Fruit from potassium-treated trees had a higher oil content than fruit from untreated trees, due both to a higher percentage of oil in the kernel and to a higher percentage of kernel. The low oil content and the accumulation of sucrose in kernels of potassium-deficient trees suggest that potassium may function in the process of conversion of sugar into oil.

3. Fruit from nitrogen-treated trees had a higher percentage of kernel and a higher protein content in the kernel than fruit from untreated trees.

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VITAMIN STUDIES OF VARIETIES AND STRAINS OF PEAS

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(WITH TWO FIGURES)

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The importance of garden peas in the diet of the population of this country is evidenced by the increasing volume of peas that are commercially canned and frozen each year. The widespread growing of peas for home use is an additional factor making the study of their nutritive value all the more important.

It has been shown that peas can supply a considerable portion of some of the vitamins in the diet. FINCKE *et al.* (**3**) pointed out that the thiamine values for 19 varieties ranged from 2 micrograms per gram, on the fresh weight basis, for Laxton's Progress to 7.1 micrograms per gram for World Record. In a study involving six varieties PEPKOWITZ *et al.* (**8**) found statistically significant differences between varieties for content of carotene and of ascorbic acid, but the magnitude of these differences was very small in comparison with other reports for thiamine content. SCOTT and BELKENGREN (**10**) have shown that the carotene content in peas varied greatly, the highest value being over four times the lowest when approximately 78 strains were compared; and that the greatest ascorbic acid content was 2.7 times the lowest. However, in some instances these reported values must be assumed to have been based on a single determination and in other cases varieties harvested in different years or different locations have been compared for varietal variation.

In the present study a number of varieties and strains of peas were grown under as nearly identical field conditions as possible over a period of several years and the variations in vitamin content studied in detail.

Methods

The peas were grown at Charleston, South Carolina in 32-foot rows, randomized blocks, or in a simple lattice with 2 to 4 replications. Approximately one pound of pods was picked at random along the entire length of the row. The peas were brought into the laboratory, shelled, and suitable samples were weighed for the various analyses.

Ascorbic acid was determined according to the method described by HEINZE *et al.* (**4**). Twentyfive-gram samples were extracted with 1% meta-phosphoric acid and the reduction of 2,6-dichlorophenolindophenol dye was determined with an Evelyn photoelectric colorimeter.

Thiamine was determined by a modification of CONNER and STRAUB'S (**1**) thiochrome procedure. Forty grams of fresh peas were blended with approximately 100 ml. of 0.04 N H_2SO_4 in the small-sized Waring blender container. The mixture was quantitatively transferred to a 250-ml. pyrex

volumetric flask with additional 0.04 N H_2SO_4 and heated in a boiling water bath for 30 minutes. The contents of the flasks were stirred by rotating the flasks every 5 to 10 minutes during the heating period. The flasks were cooled to approximately 40° C. and sufficient sodium acetate–acetic acid buffer was added to bring the contents to pH 4.5 ± 0.1. Enough taka-diastase, papain, and Pectinol enzymes were added to the buffer so that the aliquot of buffer placed in each flask would contain 0.1 gm. of each enzyme. The solutions were incubated in a water bath over night (16 hours) at 40° C., made to volume, and filtered. Aliquots of the filtrate were run through the Decalso columns at room temperature and the analysis completed as described by CONNER and STRAUB.

Riboflavin determinations for the 1943 and 1944 harvests were made on the filtrate of the enzyme digest according to the method of MACKINNEY and SUGIHARA (**7**). In 1945 the determinations were made directly on the filtrate, as described by PETERSON *et al.* (**9**). The Coleman Model 12 photofluorometer was used for reading the final solutions for the thiamine and riboflavin determinations.

Carotene analyses were made as described by WALL and KELLEY (**12**), with some modifications. Instead of adding 100 ml. of 5% sodium sulfate solution during the phasic separation, 100 ml. of 30% KOH was introduced. The KOH broke the emulsions much more rapidly, produced a sharper interface, and also removed nearly all of the chlorophyll through saponification. The final solution was read in an Evelyn photoelectric colorimeter with filter #440.

Moisture determinations were made by drying approximately 30 gm. to constant weight in a forced-draft rapid-drying oven at 70° C.

Results

ASCORBIC ACID CONTENT OF PEAS

In 1942 thirty-three varieties or strains of peas were grown in a randomized block experiment in two replicates. The varieties were harvested at weekly intervals on three different dates in April, making a total of 6 analyses for each variety. Varietal means for ascorbic acid content ranged from 29.0 to 44.4 mg. per 100 grams, on a fresh weight basis, and the differences required for significance were 4.2 mg. and 5.7 mg. at the 5% and 1% levels, respectively. Some of these varieties and strains were also analyzd in 1944 and 1945. Data for the ascorbic acid content of these 18 varieties and strains of peas in 3 different years are presented in table I.

THE EFFECT OF SIZE AND MATURITY ON VITAMIN CONTENT

In May, 1943 eight varieties of peas were harvested on the same day. The shelled peas from all varieties were in prime condition for eating. Screens were used to divide the peas of each variety into three approximately equal lots according to size, small, medium, and large. Various degrees of maturity or stages of development were represented in the three size lots

from each variety. Different screens were used to separate the three lots for the naturally small-seeded varieties, Willet's Wonder and Creole, than were used for the large-seeded varieties such as Progress. All lots of each variety were analyzed for ascorbic acid, thiamine, riboflavin, and moisture content.

In table II means on a fresh and dry weight basis are given for each of the three size lots of peas for all varieties combined. The dry matter contents for the small, medium and large size lots averaged 20.8, 25.6 and 30.5% respectively. On a fresh weight basis significant differences were found

TABLE I

ASCORBIC ACID CONTENT OF PEAS IN DIFFERENT YEARS

VARIETY OR STRAIN*	MG./100 GM., FRESH WEIGHT BASIS		
	1942	1944	1945
Alah	40.7	37.4	
Hundredfold	35.2	34.6	29.9
Progress	34.7	36.2	42.6
Progress × Giant Stride			
762A	36.4	41.6	36.2
899	36.4	39.6	33.4
770–5	35.0	41.1	.....
900	37.1	37.3	.....
298–6B–3	29.0	34.2	
898	.....	32.6	33.3
Progress × Kent Alderman			
591–3–1–1	30.7	35.9	32.9
902	37.6	41.7	.....
Progress × World Record			
557–1–13	31.2	36.5	28.4
772B	34.2	.....	31.9
Little Marvel × (Thomas Laxton × Phenomenon)			
911	36.9	36.2	32.3
781–2B	30.9	33.3	32.4
910	.....	31.0	34.0
Little Marvel × World Record 775–1B	31.5	33.5	
Phenomenon × Thomas Laxton 318–3B	32.6	.....	30.7
L.S.D. 5%	4.2	6.5	3.3
1%	5.7	8.6	4.4

* Numbers represent same strains throughout paper.

between the several lots in both thiamine and ascorbic acid contents, the small size lot averaging the highest ascorbic acid content and the large, the highest thiamine content. No significant variation occurred in the amount of riboflavin present although there was a slight trend toward a decreasing amount with increasing maturity. This trend in riboflavin content is brought out more clearly in the figures based on dry weight where a highly significant difference exists among the three sizes. On a dry weight basis both the riboflavin and ascorbic acid contents rapidly decreased as the peas matured. The observed decrease in ascorbic acid content of peas as they mature has been noted by other workers (**2, 5, 6, 8**). The thiamine content increased as the dry matter content increased, thus minimizing the differ-

ences between lots for thiamine on the dry weight basis. There was no significant difference in thiamine content between the medium and large size lots on a dry weight basis, but both were significantly lower in thiamine than the small size lot. LEE and WHITCOMBE (5) have also found that thiamine expressed on a fresh weight basis increased with increasing tenderometer readings but remained relatively constant, with the exception of the most mature lot, when expressed on a dry weight basis.

The eight varieties of peas analyzed were of different seed types: Willet's Wonder and Creole represented small-seeded types; Progress and several breeding lines, large-seeded types; while Wando and strain 802–2 were intermediate in seed type. On a fresh weight basis the mean value for

TABLE II

THE RELATION OF SIZE AND PERCENTAGE DRY MATTER TO THE VITAMIN CONTENT OF GARDEN PEAS*

	Ascorbic acid	Thiamine	Riboflavin	Dry matter
Fresh weight basis				
	mg./100 gm.	*mcg./100 gm.*	*mcg./100 gm.*	%
Small	41.7	287	131	20.8
Medium	33.0	309	122	25.6
Large	24.7	358	111	30.5
L.S.D. 5%	2.9	33	.....	2.8
1%	4.0	45	.....	3.9
Dry weight basis				
	mg./gm.	*mcg./gm.*	*mcg./gm.*	
Small	2.01	13.72	6.34	.....
Medium	1.32	11.84	4.90	.....
Large	0.83	11.60	3.69	.....
L.S.D. 5%	0.18	1.11	0.82	.....
1%	0.26	1.54	1.13	.....

* Means of 8 varieties analyzed.

ascorbic acid for all lots from the small-seeded varieties was 34.9 mg. per 100 grams; the intermediate varieties, 34.0; and the large-seeded varieties, 30.4. On a dry weight basis the mean ascorbic acid contents were 1.29, 1.55, and 1.34 mg. per gram, respectively. Because of a higher percentage of dry matter in the small-seeded varieties the slightly higher ascorbic acid value on the fresh weight basis disappeared when the values were expressed on a dry weight basis. Thiamine content appeared to be unrelated to seed type of the different varieties. The highest and the lowest variety means for thiamine content both occurred in the large-seeded types. Riboflavin content does not appear to be related to the seed type although Willet's Wonder, a small seeded variety, averaged the highest riboflavin content.

General survey for thiamine, riboflavin, and ascorbic acid content

In 1943 a survey was made on over 100 varieties or strains for general information concerning the range of content of the three vitamins, thiamine,

riboflavin, and ascorbic acid. These data are given in table III. The strains selected from the various crosses were developed by single-plant selections for 4 to 10 generations and many had been carried through addi-

TABLE III

VITAMIN CONTENT OF VARIETIES AND STRAINS OF PEAS ON FRESH WEIGHT BASIS. SURVEY 1943

VARIETY OR CROSS	SAMPLES OR STRAINS		THIAMINE	RIBOFLAVIN	ASCORBIC ACID	DRY WEIGHT
	Number		*mcg./100 gm.*	*mcg./100 gm.*	*mg./100 gm.*	%
Creole	2		328	119	35.0	25.8
Gilbo	2		285	94	28.6	25.6
Glacier	1		183	97	29.3	22.4
Hundredfold	3		268	95	25.7	22.6
Papago	1		309	112	36.9	26.7
Perfection	2		341	82	32.2	27.0
Perfectah A	2		246	99	36.8	24.2
Perfectah B	3		338	91	31.2	23.5
Perfectah, Early	2		292	90	30.3	23.6
Phenomenon	1		477	105	27.6	25.2
Progress	2		108	103	24.8	22.8
Progress*	2		303	84	34.4	23.5
Stratah	1		357	99	25.8	22.5
Wando	4		285	102	32.2	23.4
Walah	2		330	92	24.3	26.1
Willet's Wonder	5		336	131	39.7	29.2
Progress × Giant Stride	40	range	91–382	78–119	21–43	19–30
		mean	210	93	30.8	22.7
Progress × Kent Alderman	9	range	235–316	76–99	25–35	20–27
		mean	271	92	31.6	22.3
Progress × Perfection	4	range	236–309	97–112	31–39	21–23
		mean	276	106	35.8	21.6
Progress × World Record	4	range	144–234	95–112	25–32	20–25
		mean	194	106	27.2	21.9
Little Marvel × Wisconsin Early Sweet	4	range	242–323	78–93	28–31	26–27
		mean	291	86	29.0	26.0
Little Marvel × World Record	7	range	221–323	76–99	28–34	20–27
		mean	271	92	31.6	22.3
Little Marvel × (Thomas Laxton × Phenomenon)	6	range	257–309	76–99	27–32	23–26
		mean	286	90	29.1	23.9
Giant Stride × Sutton's Excelsior	2	range	366–420	97–101	20–21	22–26
		mean	393	99	20.5	23.9
Giant Stride × World Record	1		273	84	32.5	21.5

* Derived from Progress crosses and now sold as Progress.

tional generations as bulk lines. It was found that the greatest range occurred in the thiamine content, with some varieties giving indications of having 3 to 4 times the quantity of others. The ranges in riboflavin and ascorbic acid content were much smaller than for thiamine; but the two were

of nearly the same relative magnitude, the highest value being approximately twice the lowest value for each vitamin.

The thiamine content of the 40 strains from the cross Progress × Giant Stride ranged from 91 to 382 micrograms per 100 grams on the fresh weight basis. This indicated a considerable segregation for thiamine content. The Progress parent used in these crosses gave a mean value of 108 micrograms. Giant Stride does not thrive under field conditions at Charleston, hence no comparable values could be given but reports in the literature indicate that it ranks high in thiamine content. The two strains from Giant Stride × Sutton's Excelsior were very high in thiamine but were the lowest in ascorbic acid of any of the strains analyzed. The Progress × Perfection crosses gave the highest values for ascorbic acid. The range in riboflavin content among the breeding strains was less than that for ascorbic acid, but the variety Willet's Wonder contained much more than any of the breeding strains. On a fresh weight basis the three small-seeded varieties, Creole, Papago, and Willet's Wonder, had a higher mean ascorbic acid content, 37.2 mg. per 100 grams, than the intermediate types, such as Perfection, the Perfectahs, and Wando with a mean value of 32.5, or the large-seeded varieties with 27.6 mg. per 100 grams. Again these differences in favor of the small-seeded types disappeared when the ascorbic acid values were calculated on a dry weight basis. In this survey work the small-seeded types had slightly higher riboflavin values than the other types when expressed on the fresh weight basis.

Detailed studies on thiamine, ascorbic acid, carotene, and riboflavin content

In 1944 two experiments were designed to study in detail the thiamine, ascorbic acid, carotene, and riboflavin content of 34 varieties or strains. In the first experiment 22 of the earlier-maturing varieties were used. Six harvests were made over a period of 24 days, with 4 harvests of any one variety extending over 9 or 10 days. There was such a large variation associated with the wide-spread dates of harvest within single varieties, or groups harvested alike, that varietal differences were obscured in the combined harvest data. It was found that the first harvest of the earlier varieties and the last harvest of the later ones were distinctly not typical of the other three harvests of the respective varieties. By dropping the data of these atypical harvests and using only the three typical harvests of each variety, the spread of the harvest dates is reduced to 11 days and no abnormal material is included in the variety data. The means for the three harvests are given in table IV. When the data from these three harvests were analyzed statistically as randomized blocks there was no significant harvest-to-harvest variation except for riboflavin content. Each harvest came from a different field replicate, which caused a confounding of harvests with field replicates. Since the three harvests were not significantly different it may be assumed that field replicates had very little influence on the variation. The lack of influence of field replicates is brought out more

clearly in the experiment with the later varieties. The F values for varieties in the early-maturing group were significant at the 1% level for thiamine and carotene and at the 5% level for ascorbic acid and riboflavin.

Six strains from the Progress × Giant Stride cross were represented in this experiment. The range in thiamine content was from 116 to 352 micrograms per 100 gm., strains 899 and 900 having the highest values. These

TABLE IV

VITAMIN CONTENT OF 22 EARLY VARIETIES OR STRAINS OF PEAS. AVERAGE OF 3 HARVESTS. FRESH WEIGHT BASIS. 1944

VARIETY OR STRAIN	THIAMINE	RIBOFLAVIN	ASCORBIC ACID	CAROTENE	DRY WEIGHT
	mcg./100 gm.	*mcg./100 gm.*	*mg./100 gm.*	*mcg./100 gm.*	%
Alah	275	100	37.4	560	26.8
Glacier	260	92	34.3	406	20.7
Hundrefold	321	91	34.6	471	19.5
Progress	129	86	36.2	474	21.4
Progress × Giant Stride					
298–6B–3	116	87	34.2	455	20.8
762A	221	94	41.6	488	22.6
763–1–1	308	95	35.0	478	19.3
770–5–1	155	93	41.1	451	20.1
899	314	109	39.6	475	19.6
900	352	104	37.3	451	19.6
Little Marvel × World Record					
775–1–B–1	310	107	33.5	492	21.0
776–1B	209	101	38.4	477	21.0
777–1B	241	96	35.7	475	20.9
779B–1–1	283	96	35.0	454	21.4
Little Marvel × (Thomas Laxton × Phenomenon)					
781–2B	316	99	33.3	383	22.8
910	322	93	31.0	396	24.1
911	303	117	36.2	425	22.5
Progress × Kent Alderman					
591–3–1–1–1	325	78	35.9	461	21.1
902	370	98	41.7	468	18.7
Progress × World Record					
557–1–13	250	83	36.5	450	21.2
Phenomenon × World Record					
787	320	89	29.8	454	21.7
1–17*	328	91	40.0	444	19.9
L.S.D. 5%	69	17	6.5	43	2.2
1%	93	23	8.6	57	2.9

* Uncertain pedigree.

two strains were among the highest of the 40 strains from this cross analyzed in 1943. Strain 298–6B–3 had the low value of 116 mcg./100 gm. in this experiment. In 1943 the same strain had for 3 analyses a mean content of 93 micrograms per 100 gm. The Progress parent was again very low in thiamine content. Strains 781–2B, 910, and 911 from the cross Little Marvel × (Thomas Laxton × Phenomenon) gave relatively high values for thiamine. In 1943 strains from the same cross had a mean thiamine content greater than all but two other crosses.

As in 1943 the range in riboflavin content was much less than that for thiamine. Very few significant differences occurred among the strains.

The high value of 41.7 mg. per 100 gm. for the ascorbic acid content of strain 902 corresponded to a high of 35 mg. in the 1943 when it was the highest in rank among the 9 strains from the Progress × Kent Alderman cross.

The first data for carotene content in the present study are given in table IV. Although the range in carotene content was not as great as that for other vitamins a highly significant varietal or strain difference was found. This difference was not found between the strains within a cross; but highly significant differences were found between the strains of different crosses. For instance, the strains from the Little Marvel × (Thomas Laxton × Phenomenon) cross are all much lower in carotene than those from the cross Little Marvel × World Record. Highly significant differences in carotene content were found among the four commercial varieties.

TABLE V

VITAMIN CONTENT OF 12 LATE VARIETIES OR STRAINS OF PEAS. AVERAGE OF 4 ANALYSES, FRESH WEIGHT BASIS. 1944

VARIETY OR STRAIN	THIAMINE	RIBOFLAVIN	ASCORBIC ACID	CAROTENE	DRY WEIGHT
	mcg./100 gm.	*mcg./100 gm.*	*mg./100 gm.*	*mcg./100 gm.*	%
Creole	304	97	37.0	408	21.5
Miracle	438	98	36.4	714	21.3
Morse Market	341	84	31.1	462	23.4
Perfection	338	96	36.4	390	21.8
Perfectah	341	97	34.8	406	22.5
Perfectah, Early	349	89	29.6	406	21.8
Walah	328	88	28.2	338	20.8
Wando	345	96	33.4	530	22.4
Willet's Wonder	305	117	45.7	522	24.4
1–12*	447	81	19.8	430	26.6
1–19*	188	89	21.3	471	27.0
903 (Giant Stride × Progress)	407	79	22.9	453	23.7
L.S.D. 5%	44	9	5.0	71	2.7
1%	62	12	7.1	100	3.8

* Uncertain pedigree.

The second group of 12 later-maturing varieties (table V) were grown in randomized blocks in two field replicates, and two harvests were made from each replicate. All varieties from both replicates were harvested on the same day. The data were analyzed in a split plot design with replicates and varieties constituting the first split and with harvests and harvests × varieties the second split. All F values for replicates were non-significant.

The F values were significant at the 1% level for varieties for all vitamins, and for harvests for all vitamins except riboflavin. Table V gives the average values on the fresh weight basis. When the data from both groups of varieties were analyzed on the dry weight basis the F values were also significant for varieties for all vitamins.

Among the commercial varieties in this group Miracle stands out as the highest in both thiamine and carotene content. The strains 1–12 and 903

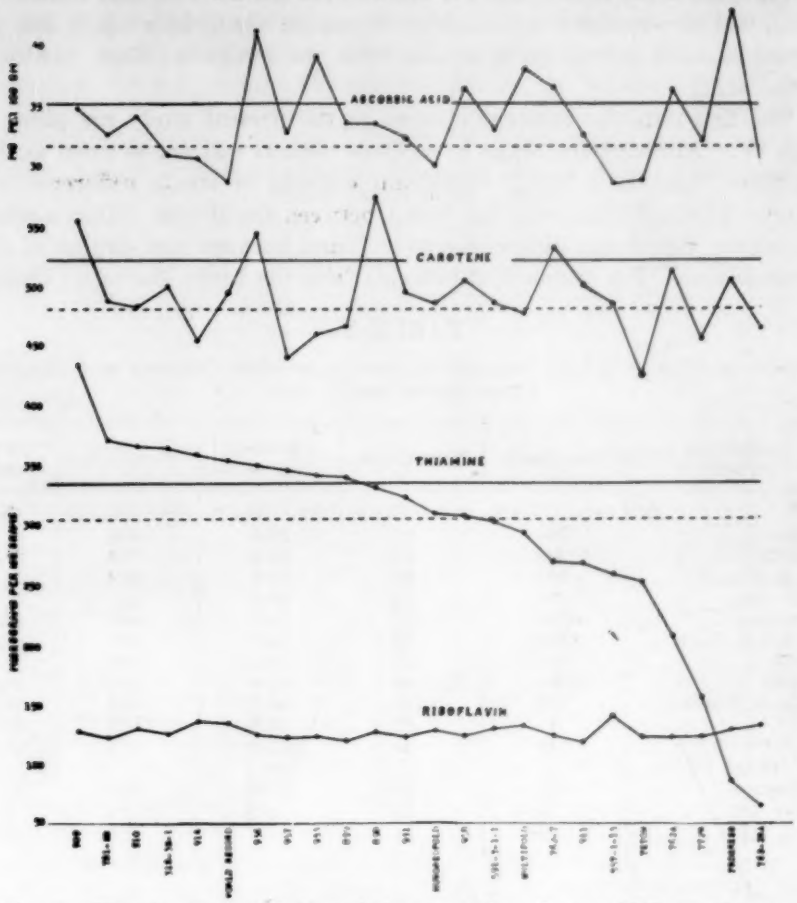

FIG. 1. Vitamin content of 24 varieties or strains of peas, 1945 experiment.

PEDIGREE OF STRAIN NUMBERS

CROSS	STRAIN NUMBERS
Little Marvel × (Thomas Laxton × Phenomenon)	909, 910, 911, and 781–2B
Progress × Kent Alderman	591–3–1–1, 931, 933, and 936
Progress × Giant Stride	762A, 762–2BA, 762–7, 898, 899, and 957
Progress × World Record	557–1–13, and 772B
Giant Stride × World Record	958
Giant Stride × Sutton's Excelsior	914
Phenomenon × Thomas Laxton	318–3B–1

The varieties having values above the solid horizontal lines are statistically significantly higher than those values appearing below the dotted lines.

were also high in thiamine content, while most of the other varieties have more of a medium range of values with the exception of the very low value

for strain 1–19. Willet's Wonder contained significantly more ascorbic acid and riboflavin than any other variety. This is consistent with its performance in 1943, when it also ranked the highest among the commercial varieties in these two vitamins.

In 1945 a simple lattice containing 25 varieties or strains and four replicates was used to give information on vitamin content as well as yield data. The individual varieties were harvested in April as they matured. All replicates of a variety were harvested on the same day. There was some difference in spread of the harvesting period for the several varieties due to slight differences in maturation rate. However, all harvests were completed within 12 days. The data were analyzed as randomized blocks except in the case of thiamine where the simple lattice design gave a sufficient gain in precision to warrant the use of values adjusted for block effect. One strain failed to produce enough to complete all analyses so it was omitted from the results presented in figure 1.

The varieties in figure 1 are arranged in a descending order according to their thiamine content. The division of the plotted values into three groups each for thiamine, for carotene, and for ascorbic acid content shows that a considerable number of varieties are significantly higher than others. If these same values are plotted on a dry weight basis for thiamine content several varieties change their relative ranking within groups, but there is only one exchange of varieties between groups and that occurs between the highest and intermediate.

The three strains 909, 781–2B, and 910, all derived from a cross involving Little Marvel × (Thomas Laxton × Phenomenon) ranked highest in thiamine content. These same strains were among the higher-ranking strains in an earlier experiment, table IV.

The strain 914 from the Giant Stride × Sutton's Excelsior cross ranked very high in thiamine content and quite low in ascorbic acid, just as it did in the 1943 survey data. It also gave quite a low carotene value in 1945.

Strains 936, 933, and 931 from the Progress × Kent Alderman cross are of interest. All three were medium to moderately high in thiamine content and the first two were definitely high in ascorbic acid content. Strain 936 appears to have combined in it the factors for relatively high thiamine, carotene, and ascorbic acid content along with an average amount of riboflavin. This strain is of particular interest in a program of breeding for higher vitamin content in peas.

Discussion

Statistically significant varietal differences were found in thiamine, riboflavin, ascorbic acid, and carotene content of peas over a period of several years. It is of interest to compare the nutritional significances of these differences. Using the data from the 1945 experiment and basing the daily allowances on those recommended by the National Research Council (1945), 100 gm. of the fresh, uncooked peas from the lowest-ranking varieties would

supply 4½% to 5% of the thiamine, 6% to 7% of riboflavin, about 14% of the carotene, and 37% to 40% of the ascorbic acid of an adult's daily requirement. In contrast, 100 gm. of the higher-ranking varieties would supply from 30% to 35% of the thiamine, 7% to 8½% of the riboflavin, about 19% of the carotene, and from 56% to 60% of the ascorbic acid. The proportions of the daily requirements actually supplied by these varieties when cooked will depend on losses in cooking. With the best available methods losses can be negligible, but under less favorable cooking conditions they may run as high as 30% of the thiamine and 60% or more of the ascorbic acid. It is readily seen that, from the nutritional standpoint, the consider-

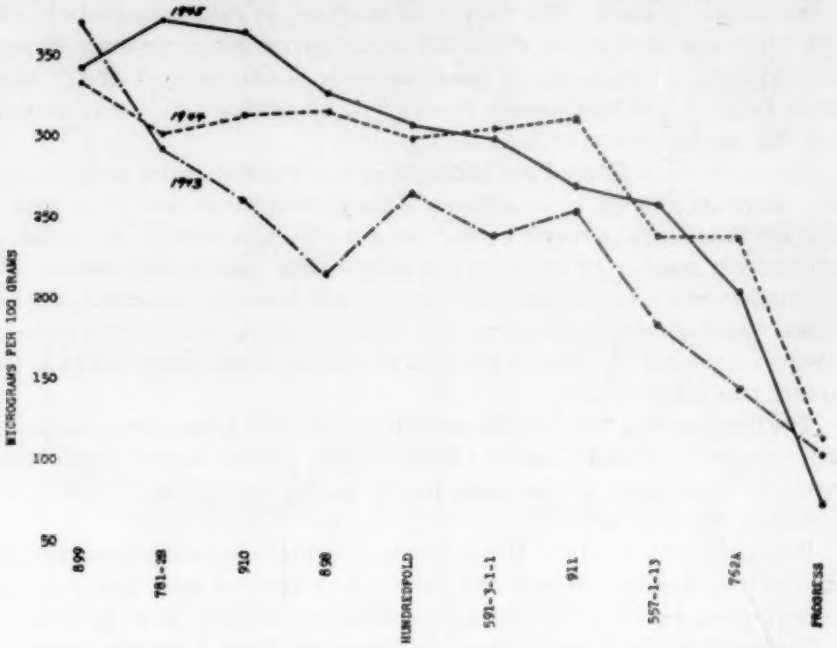

FIG. 2. Thiamine content of 10 varieties or strains of peas for a three-year period.

able variety variation in thiamine content is much more important than the relatively moderate variations in the other vitamins.

It should be pointed out that the varieties or strains represented in the 1945 experiment were all medium- or large-seeded types. If some of the small-seeded varieties such as Willet's Wonder could have been included in this study the nutritional significance of the varietal variation for ascorbic acid and riboflavin content might have been increased considerably. The difference in the time required for varieties of widely differing types to mature makes it difficult to compare any large number of varieties unless they are planted at different dates so as to bring them to the harvesting stage at approximately the same time. The early experiments in this study emphasized that the varieties being compared must be harvested over a relatively short period of time.

Ascorbic acid is the vitamin most easily lost or destroyed during cooking and preparation of the peas for serving. While the ascorbic acid content shows considerable varietal variation, the significance of this from a nutritional standpoint is thus much reduced. However, peas are generally considered a fair source of vitamin C.

The relative ranking of a few of the varieties over a period of years indicates that some varieties are quite consistent in their response insofar as thiamine content is concerned (fig. 2). Progress definitely ranked below any of the other varieties or strains in this group in each of the three years, and the breeding lines 899, 781–2B, and 910 were always high in thiamine content. Table I shows the ascorbic acid content of a number of varieties or strains over a period of two or more years. Although uniformity for ascorbic acid content from year to year is much less than for thiamine, some trends can be observed. If the eight varieties or strains for which three years' data are given are considered it will be noted that strain 762A ranked either highest or second highest in all three years. Likewise strain 781–2B was found to be near the bottom in ascorbic acid content over the three-year period. Among the strains for which only two years' data are given, 902 ranked the highest and the small-seeded variety Alah was second. Strain 902 also showed a relatively high thiamine content in 1944.

Correlation studies were made on the 1944 and 1945 data in an attempt to determine if any relationship existed between the quantities of each of the four vitamins present. In all experiments a negative correlation coefficient was obtained for ascorbic acid and thiamine content on the fresh weight basis, but the coefficient never approached a significance level in these studies.

There is a significant but not very important positive correlation between ascorbic acid and carotene content on the fresh weight basis. Correlation coefficients of 0.293 and 0.265 (significant at the five % level) were obtained for ascorbic acid and carotene content in the 1944 and 1945 data, respectively. Correlations involving all other vitamins were erratic from experiment to experiment and from year to year and very few of the coefficients approached a significance level.

In considering these strains as possible horticultural varieties it is of some importance to note their response in cold-hardiness tests. WADE (**11**) has shown that the strains 910, 898, and 899 included in this vitamin study were better than or equal to Progress in frost tolerance.

Summary

A number of varieties and breeding strains of garden peas have been analyzed for thiamine, riboflavin, ascorbic acid, and carotene content over a period of several years. Highly significant varietal differences have been found for all four vitamins.

The seed size, or stage of maturity, of the peas was found to have a significant effect on the vitamin content. On the fresh weight basis ascorbic acid decreased and thiamine progressively increased with increase in the

size of peas. On the dry weight basis the ascorbic acid and the riboflavin content decreased rapidly and thiamine remained nearly constant as the peas became more mature.

Correlation studies showed a slight significant positive correlation between ascorbic acid and carotene content. No significant relationship was found between the other vitamins.

The differences in thiamine content among pea varieties and breeding lines are large enough to be of real importance in an ordinary diet. Since thiamine content is found to be relatively consistent from year to year among varieties, it clearly merits attention in any breeding work designed to improve the nutritive value of peas.

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THE EFFECT OF AIR SUPPLY ON APPARENT PHOTOSYNTHESIS

JOHN P. DECKER

(WITH SIX FIGURES)

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It has been recognized for a long time that the rate of absorption of CO_2 by a green plant shoot or leaf enclosed in a vessel is affected by the rate at which air is supplied. Much attention has been given to the problem of maintaining a "normal" air supply; that is, a CO_2 supply comparable to that found under natural conditions. The early literature was reviewed and discussed by HEINICKE and HOFFMAN (**5**), who presented results showing that the rate of photosynthesis was below normal when the air supply was less than approximately two liters per hour per square centimeter of leaf area. VERDUIN and LOOMIS (**9**) presented results from which they concluded that photosynthesis of corn leaves "was affected surprisingly little" by 70% depletion of the CO_2 within the leaf chambers.

BROWN and ESCOMBE (**1**) presented results which they interpreted as indicating an approximate proportionality between the rate of apparent photosynthesis and the mean CO_2 concentration. They pointed out that "in all cases where the illumination of the leaf was good, although the amount of intake of CO_2 into the leaf was approximately proportional to the increased partial pressure, the photosynthetic work was always somewhat in excess of what might be expected from the increased amount of CO_2." They were obviously expecting strict proportionality and had overlooked some of the following facts. Proportionality implies a linear relationship. General linear relationship is described by the equation $y = a + bx$; but strict proportionality exists only when $a = 0$ and $y = bx$. Apparent photosynthesis becomes zero at a measurable CO_2 concentration (the compensation point); therefore $a \neq 0$, and strict proportionality cannot exist.

The data of BROWN and ESCOMBE actually contain very little information concerning the real relationship between photosynthesis and CO_2 concentration. They made only two observations on each plant, one at high CO_2 concentration and one at low concentration. Thus only two points were established for each plant, and a linear function can be derived from any two points whether established experimentally or selected at random. Further, a variety of functions other than linear can be fitted to any two points, for example, $y = ax^n$, $y = a + 1/x$.

Data presented by DENEKE (**4**) indicated that the rate of photosynthesis increased with the velocity of air over the leaves and apparently approached a maximum limit at an air velocity of approximately 100 meters per minute. He circulated air over a plant in a closed system and measured the time required for a certain reduction of CO_2 content. One set of data, his figure 8, is reproduced here as figure 1. This and similar curves suggested to

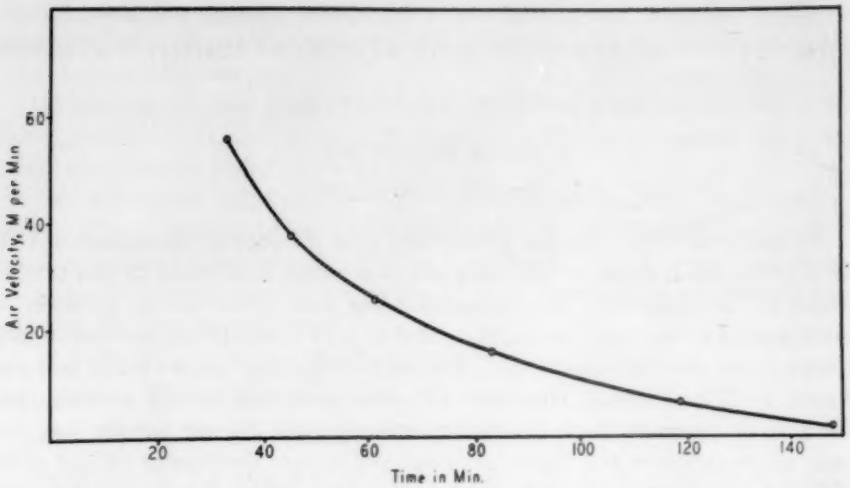

FIG. 1. Effect of air velocity on time required for a constant amount of apparent photosynthesis. Redrawn from DENEKE (4).

DENEKE that increasing velocity above 100 meters per minute would probably result in no further increase in the photosynthetic rate. However, it should be noted that rate is the reciprocal of time; that is, R = K/T where R

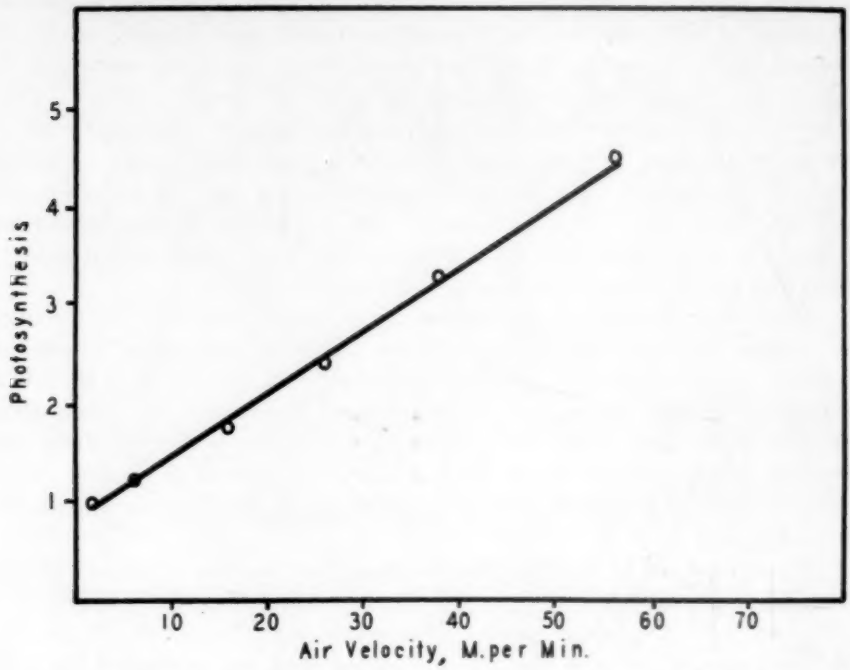

FIG. 2. Effect of air velocity on apparent photosynthesis. Recalculated from data of DENEKE (4).

is the rate of photosynthesis, and T is the time required for the absorption of a constant amount of CO_2, K. When photosynthetic rates were calculated from DENEKE's data they were found to describe a nearly straight line in relation to air velocity as shown in figure 2. DENEKE plotted T, the reciprocal of R, and the resulting curve was hyperbolic, of course. What he has interpreted as the flattening of the curve at high velocity is merely the normal shape of a hyperbolic curve.

HEINICKE and HOFFMAN (5) presented results which they interpreted as showing that the rate of photosynthesis declined more rapidly than the mean CO_2 concentration at very low rates of air supply. This implies a curvilinear relationship between rate of photosynthesis and mean CO_2 concentration. Values for mean CO_2 concentration were calculated from the data in their table 4 using the method they described, and photosynthesis was plotted over

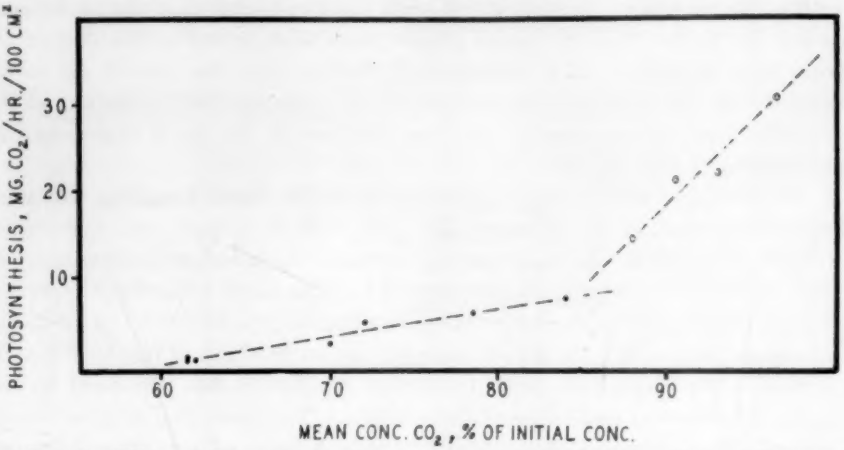

FIG. 3. Effect of CO_2 concentration on apparent photosynthesis. Data of HEINICKE and HOFFMAN (5).

mean CO_2 concentration as shown in figure 3. When the whole array is considered, a non-linear curve is suggested. It appears possible, however, that the array is composed of two sets of data describing two straight lines of different slope. It should be noted further that the four upper values were observations on single leaves and the remainder were composite measurements on many leaves. The inference of curvilinear relationship from these data is not justified.

HEINICKE and HOFFMAN stated also that the rate of photosynthesis fell rapidly when the CO_2 content was depleted more than 20%. This implies a curvilinear relationship between the rate of photosynthesis and the degree of depletion. When the data contained in their table 4 were plotted it was found, as before, that although the whole array suggested a non-linear curve, the data obviously could be separated into two groups. DECKER'S (3) conclusion concerning the non-linear effect of CO_2 depletion on the photosynthetic rate of pine was based on a misinterpretation of preliminary results.

The experimentation reported here was undertaken in an attempt to clarify partially the effect of air supply on the rate of photosynthesis of the entire shoot of a tree seedling enclosed in a chamber.

Methods

The apparatus used in measuring photosynthesis was as originally described (**3**) except that CO_2 analysis units of the type used by WAUGH (**10**) were substituted. A diagram of the apparatus is shown in figure 4. The entire top of the plant was enclosed in a transparent cylindric chamber and was illuminated from above by a battery of electric lights. Air was drawn through the chamber at a known rate, and the difference between the amounts of CO_2 entering and leaving the chamber was taken as a measure of apparent photosynthesis. A small radial-flow fan (*2*) circulated the air rapidly within the chamber. A simple test with smoke showed that the turbulence caused by the fan was very much greater than that caused by the flow of air from inlet to outlet. It is presumed, therefore, that the rate of air movement within the chamber and over the leaves was essentially constant at the different rates of air supply. Air temperature in the shoot chambers was maintained at $25° \pm 1°$ C.

The arrangement of the CO_2 analysis units for direct sampling was similar to that described by WILSON (**12**). For simplicity only one of the units is shown in figure 4. Another was connected to the blank sampling tube (*8*) and a third to the other shoot chamber (*7*). The three units were arranged in a suitable thermostatic water bath. The sample was metered (*14*) through the absorption tower (*15*) which was charged with 50 ml. of 0.0055 N NaOH solution. The change in concentration of the NaOH was measured by the change in electrical resistance of the solution. The operating routine was as follows. The tower was charged with fresh solution and was stirred for five minutes by passing the CO_2-free air stream through it. The air stream was made CO_2-free by passing it through soda-lime (*17*). The tower was then by-passed and the initial resistance reading was made. The tower was again switched into the air stream and the soda-lime tube was by-passed for exactly fifteen minutes. The CO_2-free stream was then allowed to pass through the system for an additional three minutes to sweep the remaining CO_2 into the tower, the tower was by-passed, and the final resistance reading was made. At a CO_2 concentration of 0.55 mg./l. and an air flow of 0.55 l./min. a run of 15 minutes gave a resistance change of approximately 100 ohms. An Industrial Instruments Model RC–1B bridge was used, and with a decade variable resistance coupled in series it was possible to make readings to the nearest 0.5 ohm with considerable ease and speed. Replicated tests with the three units sampling a common source of air revealed an average discrepancy between units of approximately 1%.

The sampling unit drew air from the shoot chamber at a constant rate of 0.55 l./min. The total flow through the chamber was controlled by means of another air line and flowmeter (*18*) which was calibrated over the range 0.5

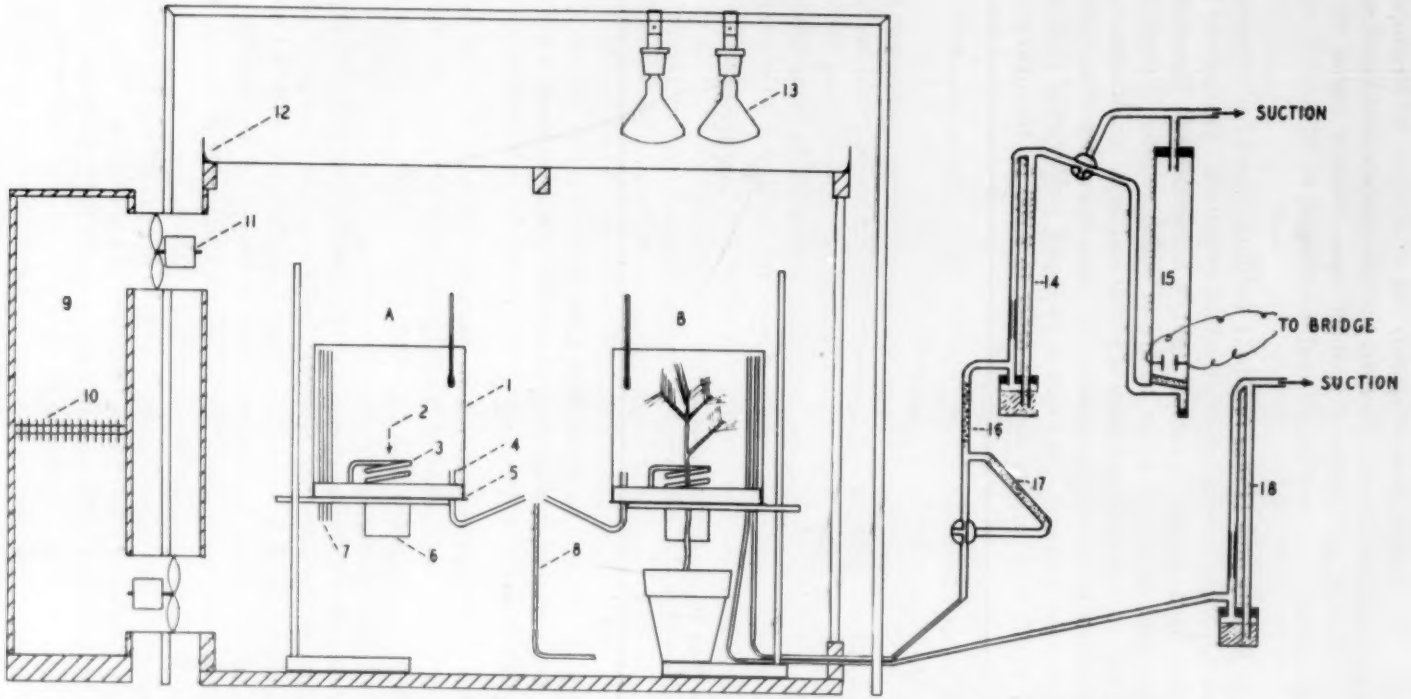

FIG. 4. Apparatus used to measure apparent photosynthesis of entire shoots under controlled conditions. Letters and numerals refer to descriptions in the text.

to 10.0 l./min. The desired rate of supply was established 20–25 minutes before a measurement was begun. Preliminary tests made at the lowest rate of air supply showed no difference between measurements begun 20–25 minutes after a change in rate of supply and those begun 45–50 minutes after the change.

Ten potted loblolly pine (*Pinus taeda* L.) seedlings which had completed the initial growth of their second growing season were used. Throughout the season they had been exposed to normal light in a greenhouse. The experiment was arranged as a 5×5 Latin square in which columns represented hours of the day, rows represented pairs of plants and days, and the five rates of flow were distributed within the square. To allow separation of any differential effect of light intensity, one plant of each pair was exposed to a light intensity of 10,000 foot candles and the other to 2,200 fc. throughout the

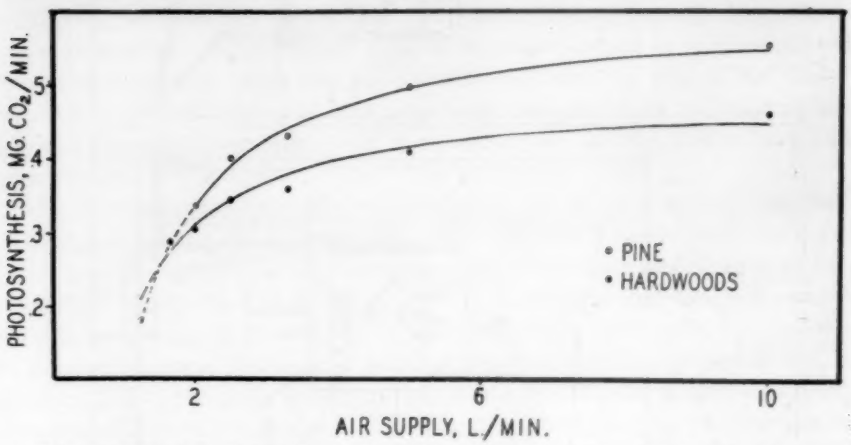

FIG. 5. Effect of air supply on apparent photosynthesis of entire shoots of tree seedlings. Data of table II.

series of flows. The Latin square design was chosen because it would reduce the chance of having the decline of the CO_2 content of air from out-of-doors through the day confounded with the effect of air supply. All plants were exposed to all rates of supply and thus the amount of tissue involved was a constant whose effect did not appear in differences between rates of supply.

The results of HEINICKE and HOFFMAN indicated that the rate of photosynthesis could probably be expected to vary hyperbolically with the rate of air supply and therefore linearly with the reciprocal of air supply. The rates of supply which were used (2.0, 2.5, 3.3, 5.0, 10.0 liters per minute) were chosen because the reciprocals were convenient values.

A second series was run using six dogwood (*Cornus florida* L.) seedlings and six tulip poplar (*Liriodendron tulipifera* L.) seedlings. These plants were in their third growing season and were kept in the greenhouse near the pine seedlings. A design similar to the previous one was used, with a 6×6 square, one plant of each species in a pair, and a sixth rate of supply of 1.67 l./min. Only one light intensity, 10,000 fc., was used in this series.

TABLE I

APPARENT PHOTOSYNTHESIS OF ENTIRE SHOOTS AT DIFFERENT RATES OF AIR SUPPLY. MG. CO_2/MIN./SHOOT

SPECIES	LIGHT INTENSITY	AIR SUPPLY, L./MIN.					
		10	5	3.3	2.5	2.0	1.67
	fc.						
Loblolly pine*	10,000	.579	.531	.464	.441	.366	.....
" " *	2,200	.530	.470	.401	.361	.309	.....
Dogwood†	10,000	.414	.371	.318	.310	.282	.260
Tulip poplar†	10,000	.519	.455	.409	.383	.333	.318

* Values are means of 5.
† Values are means of 6.

Results

Results are summarized in tables I and II. Values for mean CO_2 concentration and degree of depletion were calculated from the mean initial and final concentrations within each air supply group.

There is an obvious difference between the values for the two light intensities with pine; however, an analysis of variance (table III) revealed no differential effect of air supply with light intensity, and the two sets of data were combined for all further considerations. Similarly, no differential effect of air supply with the two hardwood species was found, and the data for the two species were combined. The statistical significance of row and column effects indicates that the Latin square design gave a real increase in precision.

Regressions were fitted to the two sets of data using 1/R (where R is the rate of air supply) as the independent variable and rate of CO_2 absorption as the dependent variable. Both were found to be linear. The analyses of variance are given in table III. The linear curves became hyperbolic, of course, when transformed from the reciprocal scale to the normal scale as shown in figure 5.

TABLE II

APPARENT PHOTOSYNTHESIS, MEAN CO_2 CONCENTRATION AND DEGREE OF DEPLETION OF CO_2 AT DIFFERENT RATES OF AIR SUPPLY

		AIR SUPPLY, L./MIN.					
		10	5	3.3	2.5	2.0	1.67
Pine	Photosynthesis (mg. CO_2/min.)	0.555	0.501	0.432	0.401	0.337	.....
	Mean CO_2 conc. (mg./l.)	0.520	0.495	0.479	0.465	0.456	.....
	% depletion	10.2	18.4	23.8	29.4	31.2	.....
Hardwoods†	Photosynthesis	0.466	0.413	0.363	0.346	0.307	0.289
	Mean CO_2 conc.	0.508	0.494	0.473	0.463	0.453	0.448
	% depletion	8.8	15.1	20.9	26.3	29.3	31.9

* Values are means of 10.
† Values are means of 12.

TABLE III

ANALYSIS OF VARIANCE

PINE			HARDWOODS		
SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Rows	4	.077205	Rows	5	.002735
Columns	4	.001496	Columns	5	.022684
Flows	4	.072026	Flows	5	.052861
Error	12	.002782	Error	20	.003940
Total	24		Total	35	
Light	1	.047678	Species	1	.106650
Light-Flow	4	.000305	Species-Flow	5	.001221
Error	20	.009930	Error	30	.002864
Total	49		Total	71	
Regression*	1	.028569	Regression*	1	.021260
Residuals	3	.000081	Residuals	4	.000195
Total	4		Total	5	
b = − .54 ± .09†			b = − .35 ± .09†		

* Regression tables are based on means of 10 and 12 observations respectively.
† Standard error of b multiplied by 5% t.

The curves of apparent photosynthetic rate plotted over the degree of depletion (not shown) appeared to be linear with essentially the same dispersion of points shown in figure 6.

Discussion

These data are in general agreement with the conclusion of HEINICKE and HOFFMAN that the effect of air supply on CO_2 absorption is curvilinear

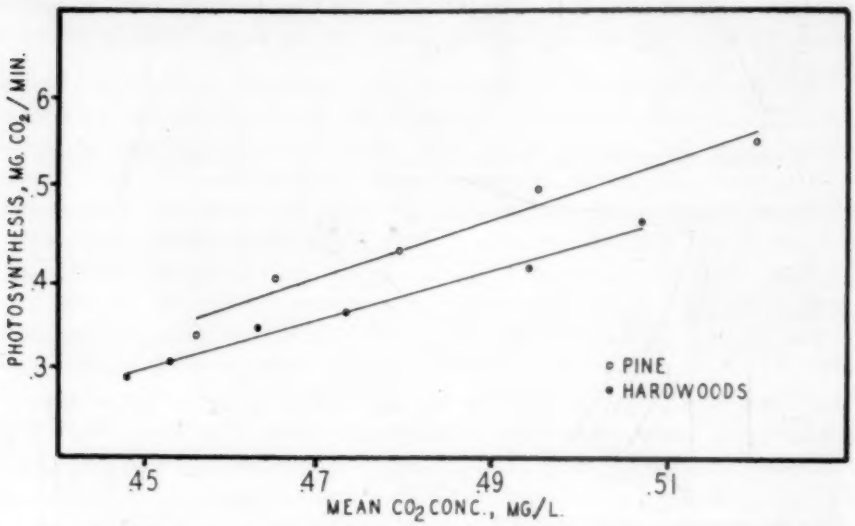

FIG. 6. Effect of CO_2 concentration on apparent photosynthesis of entire shoots of tree seedlings. Data of table II.

over the known range. There is apparent disagreement with DENEKE's data, which showed a linear relationship; however, the technique used by DENEKE differed from that used in the present study. DENEKE measured the time required for a standard depletion of the CO_2 content of a fixed volume of air in a closed system, thus the mean CO_2 content was constant for all air velocities. It appears that he measured directly the simple effect of air velocity over the leaf. In the present study the air velocity was held essentially constant and the mean CO_2 content was varied.

Considerable attention has been given in past studies of photosynthesis to the problem of maintaining a "normal" or at least known CO_2 supply. A frequent practice has been to maintain an air supply such that the CO_2 content was reduced not more than 15–20%. This practice is subject to question because it has been based on the conclusion of HEINICKE and HOFFMAN that the effect of depletion on the photosynthetic rate increases rapidly at depletions greater than 20%. There is another objection to the use of degree of depletion in attempting to evaluate or control the effect of CO_2 supply. Degree of depletion is a relative value and does not take into account the actual CO_2 pressure, thus 20% depletion when the initial concentration was 0.6 mg./l. is different from 20% depletion when the initial concentration was 0.4 mg./l. The mean CO_2 content of air as used by BROWN and ESCOMBE, that is, the arithmetic average of the affluent and effluent air, is probably a more useful value. The relationship between rate of photosynthesis and mean CO_2 concentration found in the present experimentation is shown in figure 6. It is proposed that for a given set of experimental plants a similar regression could be established, by means of which all further experimental measurements of photosynthesis could be corrected to a selected mean CO_2 concentration. The effect of CO_2 supply could thus be eliminated from comparisons between measurements. In such a regression proper account should be taken of the fact that the independent variable, x, is subject to considerable sampling error.

Apparently it is generally supposed that, because the volume percentage concentration of CO_2 in air is essentially constant at all altitudes, CO_2 is not one of the determinative factors in the altitudinal distribution of species. The writer has been unable to discover any direct evidence supporting this supposition. LUNDEGARDH (**6**) discussed CO_2 as an ecological factor but made no mention of it in relation to altitude. WEAVER and CLEMENTS (**11**) did not mention it. MEYER and ANDERSON (**7**) stated that because CO_2 concentration is nearly constant it seldom need be considered a variable in interpreting the developmental behavior of plants under natural conditions. However, the diffusion of CO_2 into a leaf is a function of CO_2 pressure rather than concentration, and the pressure of CO_2 in the atmosphere varies directly as the total atmospheric pressure. The normal pressure of atmospheric CO_2 is approximately 22.8 mm. Hg at sea level and 13.0 mm. Hg at 15,000 feet altitude. DAUBENMIRE (**2**) recognized that the partial pressure of CO_2 varies with atmospheric pressure and stated that its effect on plant growth is indirect.

From the relationship between photosynthesis and CO_2 concentration shown in figure 6 a similar relationship between photosynthesis and CO_2 pressure may be deduced, for CO_2 pressure is a direct function of CO_2 concentration at constant total pressure. These data indicate a possible differential response to CO_2 pressure of the photosynthetic mechanisms of species normally growing at the same altitude.

In general, the natural distribution of vegetation is determined by the differential responses of species to gradients in one or more environmental factors. It seems reasonable to expect, therefore, that some species characteristic of different altitudes may exhibit differential responses to CO_2 pressure which are related to distribution, and thus at times the gradient in CO_2 pressure might be one of the significant factors of the determinative complex.

Summary

The apparent photosynthetic rate of the entire shoots of tree seedlings was found to vary hyperbolically with the rate of air supply and linearly with mean CO_2 concentration over the range 0.52 to 0.45 mg./l. The possible importance of CO_2 pressure as a factor in altitudinal distribution of species is suggested.

This work was done at Duke University and was financed through a grant made by the General Education Board to the Duke University School of Forestry for a study of natural reproduction of Piedmont forests. The generous cooperation of Drs. C. F. Korstian and Paul J. Kramer made the project possible. Prof. F. X. Schumacher aided in planning the experimentation and suggested the bilinearity of the data of Heinicke and Hoffman. Dr. C. C. Camp made valuable suggestions concerning the presentation of several mathematical statements.

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ARTIFICIAL PARTHENOCARPY IN *LYCOPERSICUM ESCULENTUM*; TISSUE DEVELOPMENT

GENEVIEVE N. KING

(WITH SEVENTEEN FIGURES)

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Induction of parthenocarpy and a comparison of normal and parthenocarpic fruits was undertaken in order to determine the possible role of the embryo in the development of the fruit. There is at present a question as to whether the production of fruit is brought about as a result of growth substances from the pollen, the developing ovule, seeds, tissues of the ovary wall and placentae, or from some other region of the plant. These arguments have been reviewed by GUSTAFSON (**9, 10, 11, 12**), and it is believed that the data given below contribute further to the interpretation of this point.

Materials and methods

The Marglobe variety of *Lycopersicum esculentum* was grown under greenhouse conditions, with a temperature range from approximately 70°–85° F., and the experimental work was carried out with seventy selected plants. One group received the treatment necessary for the production of parthenocarpic fruit; the second group was kept as a control.

The seeds were planted October 6, and the seedlings were transplanted several times to secure a vigorous root system; they were kept under observation from that time until February 2, when the first treatment was applied.

Indolebutyric acid in lanolin was prepared in the manner reported by OLESON (**20**), and used in concentrations of 0.5% by weight. The flowers were emasculated, to prevent pollination, before the bud opened. Both the sepals and petals were removed with a minimum of trauma after the blossom opened in order to facilitate the application of the lanolin smear.

Applications were initially made in several ways: on the stigma, on a horizontal cut at the base of the style, or at a cut through the ovary. Parthenocarpic fruits most nearly resembling the normal fruits were produced by the treatment of the cut at the base of the style as described by GUSTAFSON (**9**). This method of treatment was used throughout the remainder of the experiment. Following the application of the lanolin-indolebutyric acid mixture, the flowers were bagged with cellophane envelopes to prevent any contamination by pollen, although it has been reported that the pollen becomes inactivated when in contact with lanolin mixtures containing some growth-promoting substance. Treatments were continued on the blossoms as they opened through February and March.

Beginning March 2, following the above treatment, flowers were cut below the abscission layer of the pedicel and collected at two-hour intervals for a period of twenty-four hours; further collections were made at 24-hour

intervals over a period of two weeks to obtain samples showing the changes as they took place following the application. Samples were also collected of comparable stages of the flower following pollination. Records were made of the general development of the treated and control plants as well as of the normal and parthenocarpic fruits. These records included the rate of fruit development following pollination and the treatment with lanolin smear, ultimate size and weight of the fruit, increase in size of the pedicel, and the premature abscission of flowers and fruits.

All tissue samples were preserved in formalin-acetic-alcohol and embedded in paraffin following dehydration with n-butyl alcohol. Sections were cut 10–12 microns in thickness and stained with safranin and fast green. Serial sections of important developmental stages were cut in both transverse and longitudinal planes to follow the sequence of events in the early development of normal and parthenocarpic fruits.

Data and discussion

The artificial induction of parthenocarpic fruit in the tomato by the use of a 0.5% by weight of indolebutyric acid in lanolin at the base of the style parallels the work of SCHROEDER (**21**) and HOWLETT (**16**). The development of the fruit was much more rapid following the treatment with the lanolin smear than as a result of natural pollination but the ultimate weight and size of the fruit were approximately the same. The parthenocarpic fruits produced were more solid, and the percentage of set of fruit was as great, or greater, than that of the normal fruit. Larger fruits were produced if the treatment followed partial pollination. The percentage of fruit-set was increased above that of the controls if repeated applications of the stimulant were made at short intervals. In agreement with the results obtained by HOWLETT (**16, 17**), parthenocarpic fruits were produced on the same plants with fruits producing seeds and their growth was not impaired if repeated indolebutyric acid treatments were given. Histological studies were made of the early stages of flowers and fruits of the normal and treated plants in order to compare their development.

The development of flowers and ovules has been described by COOPER (**4, 5**). The Marglobe tomato has five or six locules, usually six, and free central placentation (fig. 1). It is impossible to get an accurate idea of the developments within the embryo sac with sections 10–12 microns in thickness such as were used in this investigation. It is possible, however, to know the condition of the ovule in the flower at the time of pollination (fig. 2) and at the time of the application of the lanolin-indolebutyric acid mixture (fig. 4) and thus to follow the subsequent changes.

The tomato fruit consists of pericarp, ovules, and placental tissue. The greatest changes during fruit development take place in the ovules and the placentae. The development has been accurately described by HAYWARD (**14**) and general descriptions of parthenocarpic fruits have been given by several authors (**5, 6, 7, 9, 10, 11, 12, 13, 16, 20, 21, 22**). The development

of the placental tissue of the parthenocarpic fruits, however, has not been stressed. A critical study of the parthenocarpic sections showing placental tissue at the time of pollination (fig. 1), one day after treatment (fig. 3),

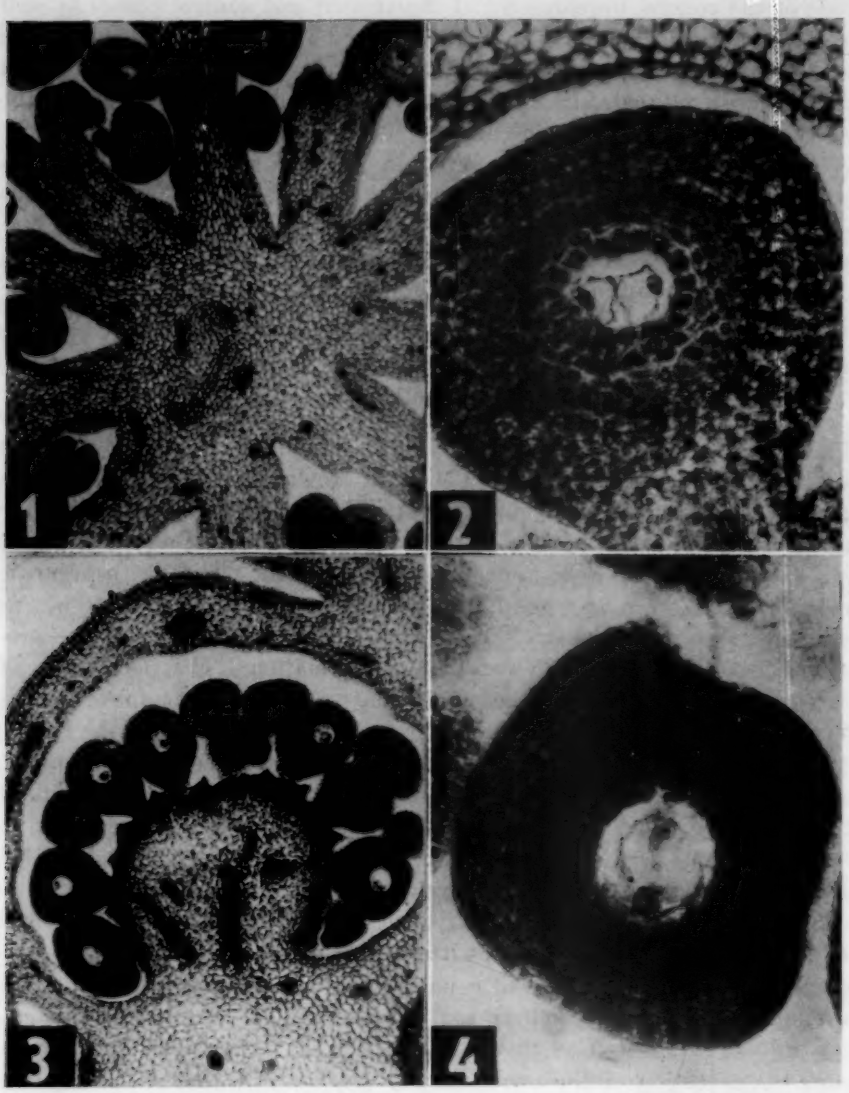

FIG. 1. Transverse section through the ovary of the flower showing free central placentation. FIG. 2. Section through the ovule at the time of pollination. FIG. 3. Longitudinal section through the ovary one day after treatment, showing placental tissue. FIG. 4. Section through the ovule one day after treatment.

and ten days after treatment (fig. 5), show the marked overgrowth of this tissue which in the mature fruit crushes the ovules against the ovary wall. This development is brought about by an increase in cell number and cell

size as stated by GARDNER and KRAUS (5). The general pattern of cell shape and cell size will vary among plants, due apparently to genetic influence (15). In fruits produced by the treatment following a horizontal cut through the young ovary (fig. 16), the growth of the placentae takes

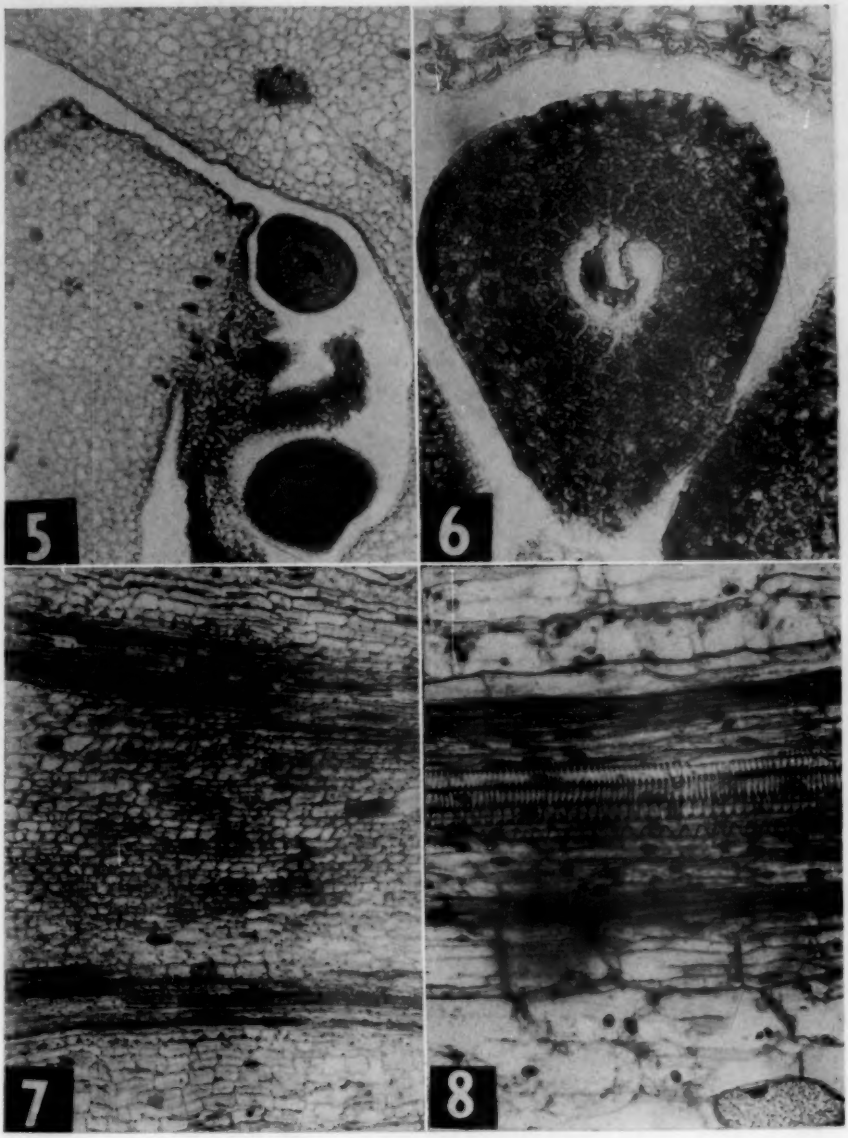

FIG. 5. Longitudinal section through the ovary ten days after treatment, showing placental tissue. FIG. 6. Section through the ovule ten days after treatment, showing the collapsed embryo sac. FIG. 7. Longitudinal section through the pedicel, directly below the receptacle region of the flower approximately ten days after pollination. FIG. 8. Same as figure 7, showing details of vascular tissue.

the form of finger-like projections. The site of ovule attachment may be observed as scar tissue on the surface of these projections. The unfertilized ovule usually ceases its development early but if there is enlargement, it is due to the slight development of integument and nucellar tissue (5). The

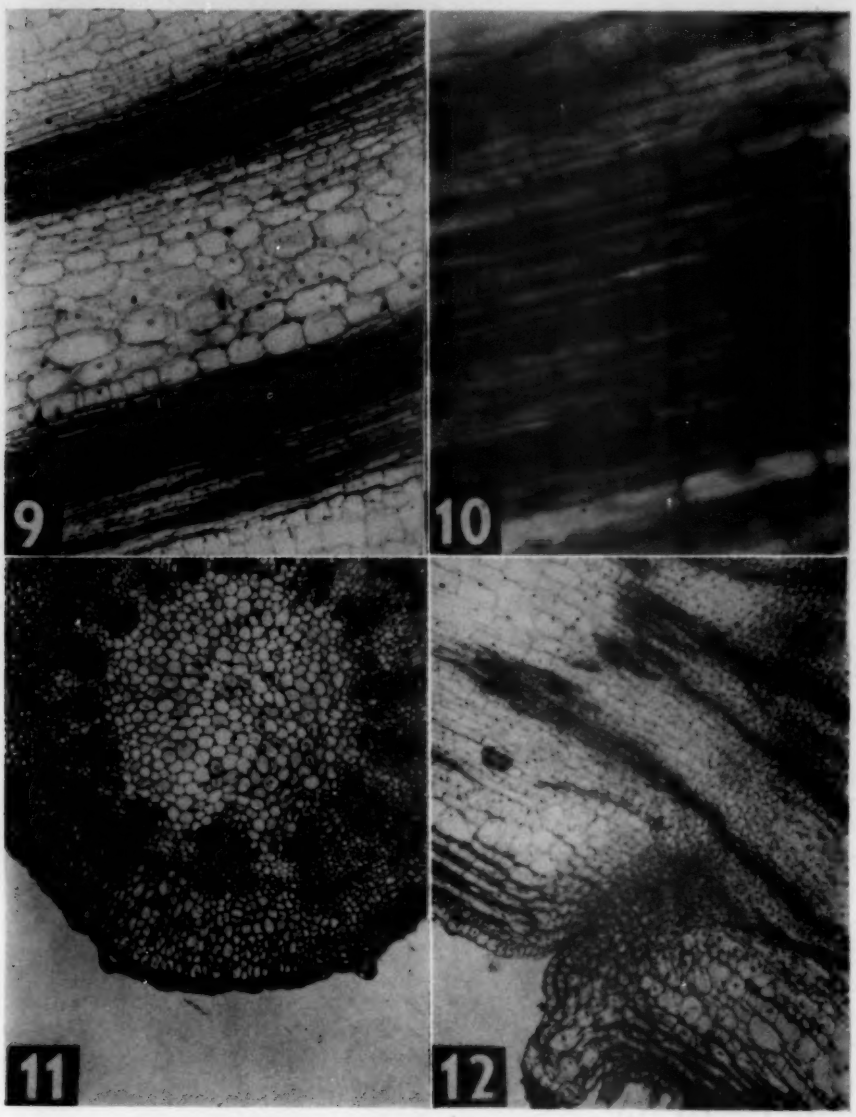

FIG. 9. Longitudinal section through the pedicel, directly below the receptacle region of the flower, approximately ten days after treatment. FIG. 10. The same as figure 9, showing details of vascular structure. FIG. 11. Transverse section through the abscission region of the pedicel, of the control showing the meristematic cells of the cortex. FIG. 12. Longitudinal section through the abscission region of the pedicel, of the control showing the meristematic cells of the cortex.

megagametophyte collapses a few days after the treatment with indolebutyric acid as noted by BORTHWICK, HAMNER, and PARKER (**2**).

The influence of the lanolin-indolebutyric acid mixture extends through the fruit into the pedicel and prevents abscission of flower and fruit. There are many references in the literature on this point (**2, 5, 6, 7, 8, 12, 16, 19**). Further data have been given by GARDNER and MARTH (**6**) who state that flower buds could be stimulated to renew growth even after the pedicel had begun to turn yellow, and that this increase of growth in the pedicel in the Solanaceae was even more rapid than that following pollination. This rapidity of effect would be expected in consideration of the rapid response secured by ZIMMERMAN, HITCHCOCK, and WILCOXON (**22**) in their work with

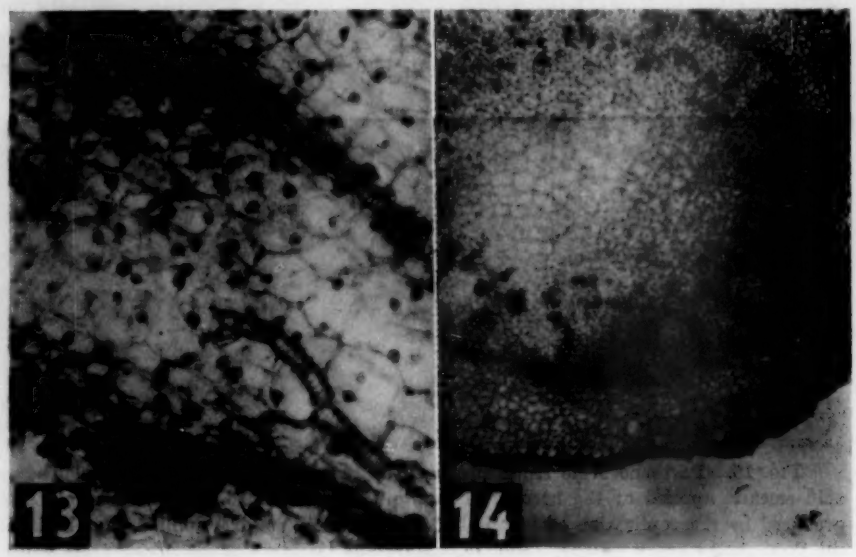

FIG. 13. The same as figure 12, showing details of vascular tissue. FIG. 14. Transverse section of the abscission region of the pedicel, of the control showing the meristematic cells of the pith.

the tomato. A comparison of the sections of the pedicel of the normal and parthenocarpic fruit taken directly below the receptacle region (figs. 7, 8, 9, 10), show a distinct difference in the amount of vascular tissue. The pedicel of the parthenocarpic fruit has more secondary tissue and larger cells in the pith and cortex. These data also agree with the work of KRAUS, BROWN, and HAMNER (**18**) as to the effect of growth-promoting substances on stem tissues. Contrary to the above results, GARDNER and KRAUS (**5**) report that it is not possible to detect any direct outstanding differences in the vascular system of fruit or pedicel in comparing normal and parthenocarpic fruit. The difference noted by the writer may be due to the repeated applications of lanolin-indolebutyric acid mixture on the base of the style. In the abscission region of the control the meristematic activity

is noticeable in both the cortex and pith regions and there is a separation or branching of vascular strands (figs. 11, 12, 13, 14). The difference in the pedicel of the normal and parthenocarpic fruits is not restricted to the internal anatomy but may be observed in the external appearance (figs. 15, 16, 17) as an increase in thickness over that of the normal fruit extending from the receptacle through the abscission region.

The theories of the causal mechanism of fruit development are reviewed by GUSTAFSON (**9, 10, 11, 12**). From the experimental work discussed, it is evident that the ovary wall is not important to any great extent in the production of growth-promoting substances which result in the development of fruit. When ovules are removed, fruit will not develop unless the cavity which is left is filled with a mixture containing a growth-promoting substance. The question arises as to whether the placentae, ovules, or embryo are responsible for the development of the fruit. In parthenocarpic fruits

FIG. 15. Parthenocarpic fruits produced by the application of lanolin-indolebutyric acid smears, applied at the base of the style (×2). FIG. 16. Parthenocarpic fruits produced by the application of lanolin-indolebutyric acid smears, applied on a horizontal cut through the ovary (×0.5). FIG. 17. Marglobe tomatoes as developed from pollination followed by fertilization (×0.33).

which are seedless and in which ovules do not develop, it is evident that the development could not have been brought about by the ovules or the embryo sac. In the work of TUKEY (**22**), the other possible alternative is discussed and his data show that the abortion of the embryo may cause the early ripening of the fruit. Parthenocarpic tomato fruits may appear in the field at certain seasons of the year and it is believed that such cases may be explained on the basis that pollination has occurred and that the pollen tube with its contents in the style is responsible for the development of the fruit even though fertilization has not occurred (**13**). GUSTAFSON (**9**) states that the high auxin content in the flower bud stage is responsible for parthenocarpy. It is evident from the experimental work discussed by the same author that there is an increasing gradient in auxin concentration from the ovary wall to the developing seed. If the region of greatest concentration in the developing fruit is eliminated, it is logical to assume that

the region next in concentration would take over the control of mobilization of materials into the fruit. Such regions in order of decreasing concentration would be ovules, placentae, and ovary wall. The production of parthenocarpic fruit when the ovules fail to continue their development, as described in this paper, shows that the placenta itself may be stimulated to fruit development without the influence of the developing ovule.

The production of induced parthenocarpic fruits in which the ovules fail to continue their development beyond the stage reached at flowering would lend support to the theory that the ovule is not essential for fruit production. Other tissues of the fruit itself, such as the placentae, may be either the source of the stimulus for fruit development or the loci at which that stimulus exerts its effect. It is possible that the auxin gradient in fruit is determined by the tissue pattern established by the flower or floral primordia.

It is interesting to note in connection with the abscission layer that the vascular strands in that area are smaller; in many cases only a single row of tracheal elements. The tracheids retain their nuclei and cytoplasm over a much longer period than previously believed. Such conditions show a distinct adaptation of the structures of the abscission layer to its function which once again emphasizes the dynamic nature of plant tissues.

Summary

1. The parthenocarpic fruits produced by the application of 0.5% indolebutyric acid in lanolin were seedless, solid, juicy, and as large as the normal fruit. The yield was as high or higher than the normal fruit and when produced on the same plant with the normal fruit their development was not affected.

2. The ovules of the parthenocarpic fruit failed to continue their development to any degree after the stage at which the flowers were treated.

3. The embryo sac of the partially developed ovules of the treated flowers collapsed and the ovules were crushed against the ovary wall by the overgrowth of the placental tissue.

4. There was an excess tissue development in the pedicel of the parthenocarpic fruit as shown by cross sections, and this increase in the thickness of the pedicel is due to an increased amount of secondary tissue.

5. Parthenocarpic fruits produced on the same plant with normal fruits were as large as those produced on separate plants.

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THE POROMETER METHOD FOR THE CONTINUOUS ESTIMATION OF DIMENSIONS OF STOMATES

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(WITH FOUR FIGURES)

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In studies concerning the movement of gases and vapors into and out of the leaf, it is frequently necessary that the dimensions of the stomates be determined. Such information may be more useful if it consists of a continuous record of the stomatal aperture rather than a series of periodic measurements. Two methods are generally used for the procurement of such continuous data; direct microscopic measurement and the porometer.

Direct microscopic measurement of the dimensions of the stomates has been considered the most accurate method by many workers. There are, however, several difficulties which make accurate measurements by the microscope difficult. For certain leaves, under some conditions, accurate measurements can be made only with extreme difficulty. For other leaves, the microscope cannot be used at all. The method is not well adapted for continuous observation, because of the strain imposed upon the worker and because of undesired changes induced through mechanical manipulation and the use of light. Also, since only a few stomates can be measured at one time, the question arises as to whether they constitute a representative sample or reflect correctly the average stomatal aperture. However, when employed for the determination of the dimensions of the stomates at a particular instant, a high degree of accuracy may be achieved.

The porometer is a device used to measure changes in the rate of flow of air through the leaf. A cup or tube is attached to the leaf and the pressure of the air within the cup is either raised or lowered with respect to normal air pressure. It is assumed that the changes in the rate of air flow are directly proportional to the changes in the dimensions of the stomates.

The porometer was first proposed by Dutrochet in 1832, according to DARWIN and PERTZ (**3**). In 1876, Muller constructed an apparatus employing this principle which proved too complicated and cumbersome for general use and acceptance. The modern design for the porometer was developed by DARWIN and PERTZ (**3**). Improvements were made on this type of porometer by KNIGHT (**7**), and by LAIDLAW and KNIGHT (**9**) who devised a constant flow aspirator and made the process self-recording. Other porometers were devised by BALLS (**2**), and by JONES (**6**). A porometer which measures changes in the rate of flow of air through the leaves in terms of pressure was devised by GREGORY and PEARSE (**4**). This method uses small rates of air flow and low pressures. It will be described in detail in another section.

Numerous criticisms have been leveled at this method, many of which are no longer valid in the light of present information. LOFTFIELD (**10**) considered that until a "comparison has been made between a series of direct observations upon the conditions of the stomata and porometer readings made at the same time—its reliability is questionable."

ASHBY (**1**) compared readings of a porometer with simultaneous measurements from epidermal strips. He concluded that with large apertures the two methods are essentially measuring the same thing but that with small apertures the porometer method is more than ten times as sensitive. He also found the error of replication of the porometer to be less than 3% while that of the strip method was over 20%. MASKELL (**11**) compared the porometer rate with the area of the stomates determined from impressions. Although he made only a small number of comparisons, his results indicated a linear relation between porometer time and the area of the stomates.

In a study of the effect of certain gases on permeability of the leaves, PAWLENKA (**13**) made a great many simultaneous measurements of the stomates using the porometer and the strip method which were reinforced by direct microscopic observations. Although not intended as a check upon the porometer, it affords the most extensive comparative investigation yet reported. Invariably, there was a direct relation between the porometer time and the area of the stomates as determined by the strip method which was constantly checked by direct microscopic observation. HARTSUIJKER (**5**) after a critical study of the most important methods to determine the dimensions of the stomates, concluded that there was a direct relation between the porometer time and the area of the stomates as measured by direct microscopic observation. He considered that the porometer was an extremely useful device for the quantitative investigation of the changes in the dimensions of the stomates.

The rate of the passage of air through the leaf is also influenced by the resistance of the cells of the mesophyll. If the intercellular volume were to change appreciably during the course of an experiment, the porometer rate would be a reflection of this change as well as of changes in the dimensions of the stomates. NIUS (**12**) investigated this question and concluded that such changes actually occurred. If Nius' conclusions are correct, a serious and uncontrollable error would affect the accuracy of the porometer method. This question was reinvestigated by HARTSUIJKER (**5**) who concluded that Nius' methods contained so many errors that his results were not valid. By the use of improved methods of a known small error, he showed that the effect of changes in the intercellular volume is extremely small. The writer has repeated these experiments of Hartsuijker and confirmed his results

KNIGHT (**8**) showed that some stomates tend to close when air is drawn through them continuously. This difficulty is associated with the use of high pressures and large displacement of volume. If the pressure used is small and the volume of air drawn through the leaf reduced to a minimum,

stomatal action appears to be unaffected. The writer has obtained records from plants growing under controlled conditions in which the stomates, as measured by the porometer, were continuously and evenly open for more than twenty-four hours at a time. It would appear that the method itself, even though used continuously, does not induce closure.

The porometer method has several decided advantages when compared to other methods. All measurements are based on the action of a large number of stomates. For comparable results to be obtained by the direct methods, several thousands of stomates would need to be measured at each reading. Since the porometer reading is determined by the average aperture of the stomates, there is a small standard error of replication. The writer has tested this point repeatedly and has found the error to be within 3%. The small standard error implicit in this method permits accurate measurement of small changes in aperture. A further important advantage is that direct, continuous records of the movements of the stomates may be obtained under either natural or controlled conditions. The writer has obtained continuous records for periods of more than four months from plants growing out-of-doors.

The porometer method for determining the apertures of the stomates is apparently accurate, and suitable for continuous operation. It is felt that the objections which have been raised against this method, are either based on insufficient data or have been met by improvements in the method. It is considered that for most studies dealing with the effects of environmental factors upon the activities of the guard cells, the use of the porometer affords the possibility of accurate continuous quantitative measurement.

The resistance porometer

Since the porometer method has so many advantages, considerable attention was given to the development of a simple, reliable recording instrument. The resistance porometer, divised by GREGORY and PEARSE (**4**) is considered the most useful for precise continuous measurement of the aperture of the stomates under practical conditions. The apparatus which they used in recording the behavior of the porometer is both cumbersome and expensive and limits the usefulness of the porometer to the laboratory. A recorder was devised by the writer which is simple to operate and inexpensive to construct and has the additional advantage of requiring a very low pressure differential for its operation.

The method may be explained by reference to figure 1. A leaf, which when attached to a leaf cup behaves as a variable resistance, is connected through a fixed capillary resistance to a vacuum line. A tambour which is a flanged metal cup approx. 1″ in diameter and $\frac{3}{8}$″ deep, sealed at the top with a thin rubber membrane, is inserted between the leaf cup and the fixed resistance to respond to changes in air pressure in the system. A constant reduced pressure equivalent to about one cm. of water is applied to the open end of the fixed resistance. The magnitude of this pressure is controlled by

the depth to which the spill-over tube is inserted into the liquid in the pressure regulator.

If more air enters through the leaf into the leaf cup than is removed through the capillary resistance, the pressure within the system increases somewhat, causing the tambour membrane to rise. If less air enters through the leaf than is removed through the capillary resistance, the pressure decreases and the tambour membrane falls. Since the stomates control the passage of air through the leaf, the pressure changes reflect the movements of the stomates. As the stomates close, entrance of air is restricted and finally stopped, the pressure decreases and the tambour falls. As the stomates open, air enters the system, the pressure increases, and the tambour rises. The movements of the tambour membrane are transmitted by a

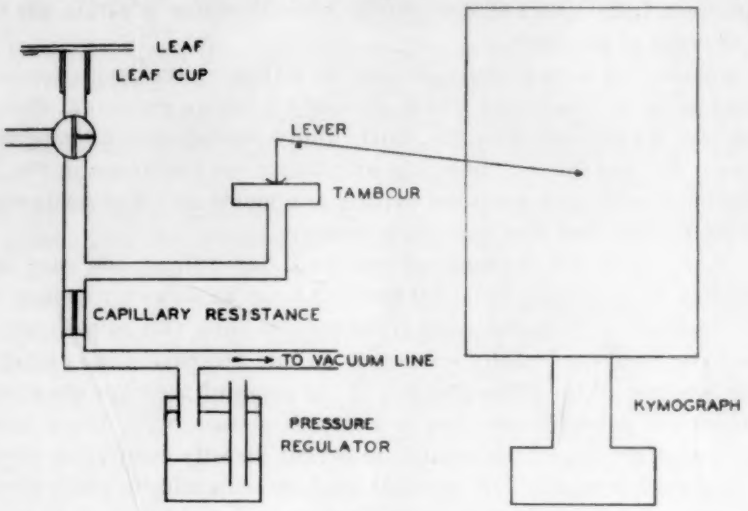

FIGURE 1

FIG. 1. Diagram of resistance porometer showing relation of various parts of system.

multiplying lever to smoked paper on a kymograph drum and a record is thus obtained.

The proper size of capillary resistance can only be ascertained by experimenting. If, as suggested by GREGORY and PEARSE (4), the thermometer tubing used for resistance is cut into segments with lengths in the ratio of 1:2:2:5:10, then a range of resistances from 1 to 20 can be obtained. Student thermometers, cut to a basic unit of 1 cm., were used. A glass T-tube was attached to both ends of each capillary segment. The tubes were then connected by rubber tubing to form a chain consisting of a piece of capillary alternating with a T-tube. Such an arrangement leaves an open end of a T-tube on either side of each piece of thermometer tubing. If these open ends are then joined by a piece of rubber tubing, they form a series of by-passes around each piece of thermometer tubing. However, if one of the

by-passes is closed with a metal clip, the air must pass through the capillary tubing. Thus by regulating the air through either the by-pass or the capillary tube, the total resistance in the circuit may be changed from 1 to 20 without allowing any outside air into the system. If too large a resistance is used, the tambour will register normal pressure while the stomates are still partly closed because air is not being removed as rapidly as it enters. If too small a resistance is used, the tambour will register reduced pressure even when the stomates are wide open because air is being removed more rapidly than it enters. The system is most sensitive when the fixed capillary resistance is equal to the resistance offered by the leaf, but since the leaf resistance varies greatly in the course of a day such a relation cannot be maintained. The proper resistance is one with which the change in pressures between fully open and completely closed stomates is within the total pressure range of the system.

In practice this is most easily attained by adjusting the resistances while the plant is under conditions which generally promote maximum stomatal opening; i.e., a high light intensity, fairly high humidity, and a temperature of between 25° and 30° C. Starting with maximum resistance in the line, the resistance is reduced, until the writing arm moves and then holds steady indicating slightly less than maximum pressure.

The leaf cups which are made of thin wall glass tubing, can vary somewhat in size, cups ranging from 0.9 cm. to 2.5 cm. in diameter having been used successfully. As many as six of the smaller cups, two on each of three plants, have been successfully connected on one recorder, thus giving the average behavior of the three plants. If the edges of the cups are ground, the cement will adhere better than to a smooth surface. The lower ends of the small cups are drawn out enough to permit directly connecting them to small bore rubber tubing. A one-hole stopper containing a piece of glass tubing is inserted in the bottom of the large cups to enable them to be connected to tubing. The volume of the cups and connecting tubing should be kept as small as possible to limit the volume of air in the system, thereby decreasing thermometer effects and increasing the speed of reaction to changes in stomatal aperture.

It is frequently difficult to seal leaves to the leaf cups. Numerous adhesives have been tried with varying success. Anderson found a mixture of approximately 1 part beeswax, 2 parts rosin, 2 parts lanolin and 1 part grafting wax fairly satisfactory for attaching leaf cups to cotton leaves. The writer found that if latex is first exposed to the air until it thickens to the consistency of thick cream it can be used to form a tough, air-tight and non-toxic seal. The edge of the leaf cup is covered with a layer of latex and held in contact with the leaf by means of a light weight until the latex is dry. Leaves of plants growing out-of-doors have been sealed to leaf cups with latex for more than a month without injury. Of course, the leaves must be supported in such a way that movements caused by wind will not

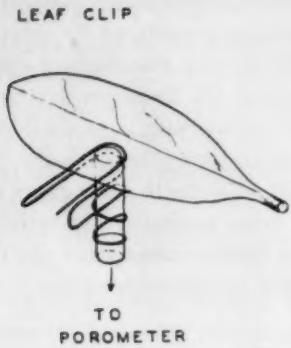

FIG. 2. Leaf clip for fastening leaves to porometer cup.

tear them loose from the rigidly fastened leaf cups. In addition to fastening the branches to supports, it is often helpful to hold the leaf in contact with the cup by means of a wire support as shown in figure 2.

The fulcrum of the magnifying lever was constructed from parts obtained from a cheap alarm clock. The clock was dismantled and all the gears except the balance wheel removed. Wooden dowels whose diameter was equal to the inside diameter of ordinary drinking straws were notched and cemented on opposite sides of the balance wheel similar to two spokes projecting from the same diameter. A straw with a gravity writing point was attached to one of the dowels. A weighted straw was attached to the other dowel to act as a counter balance. A hole was drilled in one of the spokes of the wheel into which could be inserted a free swinging arm which

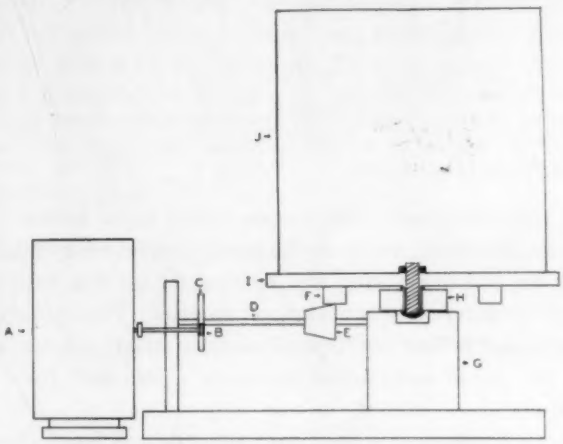

FIG. 3. Diagram showing construction details of kymograph. A, Big Ben clock; B, eight-tooth gear soldered to hour arm; C, forty-eight-tooth gear; D, connecting rod of capillary glass tubing; E, Rubber stopper; F, sponge rubber flange with circumference of four times E; G, kymograph support; H, roller skate bearing; I, kymograph drum platform; J, kymograph drum.

rested on the tambour. Two bolts were soldered to the top of the frame of the clock. The frame was then suspended by means of two screws from a metal L. By adjusting the screws, the writing point of the lever may be set to rest along any portion of the kymograph drum.

The construction of the kymograph is explained in figure 3. Although the construction was simple and very inexpensive, requiring only one ball-bearing roller skate wheel in addition to some gears obtained from the alarm clock, the kymograph was quite accurate and reliable. Eight kymographs were constructed, no one of which varied more than five minutes in twenty-four hours. Regular glazed kymograph paper is most suitable although

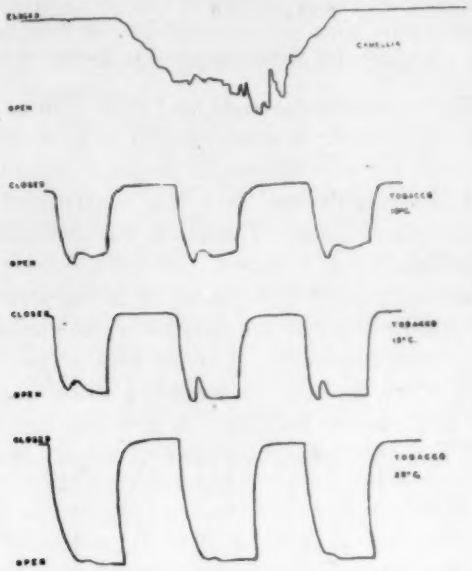

FIG. 4. Tracings of actual records; (a) twenty-four-hour record of Camellia under natural conditions; (b) records of tobacco under successive four hour light and dark periods at three different temperatures.

legible records were obtained using paper taken from better grade magazines. The drum, on which has been fastened a sheet of kymograph paper, is smoked over an ordinary wing tip burner whose gas supply has been bubbled through a wash bottle containing benzene. This procedure gives a very smoky flame and is best performed under a hood. After a record has been obtained, the paper is removed from the drum and fixed in a dilute solution of shellac.

Figure 4 shows tracings of actual records obtained from plants growing under both controlled and natural conditions. The results of scores of such records will be published in a later paper.

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CULTURE CONDITIONS AND THE DEVELOPMENT OF THE PHOTOSYNTHETIC MECHANISM. V. INFLUENCE OF THE COMPOSITION OF THE NURIENT MEDIUM

JACK MYERS

(WITH TWO FIGURES)

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Significant effects of light intensity on the cellular and photosynthetic characteristics of *Chlorella pyrenoidosa* have been described in preceding papers (**6, 7**). These studies required a more or less arbitrary choice of other culture conditions, *e.g.*, light quality, temperature, carbon dioxide concentration, composition of the nutrient medium, and the population density at which cultures are maintained in the continuous-culture apparatus. Considered herein are the effects of various changes in composition of the nutrient medium and in the carbon dioxide concentration. The problems are: (1) whether the concentrations of various components of the medium are optimal and (2) whether there may be significant effects of small changes that might be induced as a reaction in response to other variables.

This investigation depends upon the use of a continuous-culture apparatus, previously described (**5**). The data to be presented demonstrate the usefulness of the apparatus in studies on nutrient deficiencies in microorganisms.

Procedure

Chlorella pyrenoidosa was grown in a modified Knop's solution in two units of the continuous-culture apparatus. The Knop's solution contained 0.010 M $MgSO_4$, 0.012 M KNO_3, 0.009 M KH_2PO_4, 13.3×10^{-5} M $Fe_2(SO_4)_3$, 56.0×10^{-5} M sodium citrate, and 1.0 ml./l. each of the A_5 and B_6 solutions of ARNON (**1**). The A_5 solution contained 0.5 parts per million B, 0.05 p.p.m. Mn and Zn, 0.02 p.p.m. Cu, and 0.01 p.p.m. Mo; the B_6 contained 0.01 p.p.m. each of V, Cr, Ni, Co, W, Ti. The medium was prepared from stock solutions of the major salts purified by the adsorption procedure of STOUT and ARNON (**8**) and adjusted to pH 5.0.

The culture conditions adopted for reference were the same as those used previously, *i.e.*, a population density of 0.6–0.7 cmm. cells/cc., 4.4% carbon dioxide, and a temperature of 25° C. A light intensity of 25 fc as provided by tungsten lumiline bulbs was chosen because, as shown by the previous work, this gives cells with a maximum capacity for photosynthesis.

In the continuous-culture apparatus a photometric device controls the addition of fresh medium to the culture suspension at just such a rate that the density of population is maintained approximately constant. Samples of the culture suspension are harvested at 24-hour intervals, leaving a known amount of inoculum for the next interval. Because of the characteristics

of the apparatus, logarithmic growth is continuously maintained. Rate of growth is then expressible in terms of the constant k in the equation $\log \frac{N}{N_0} = kt$. In applying the equation N_0 is expressed as the milliliters of inoculum at the beginning of each interval and N as the total milliliters (ml. inoculum + ml. sample) at the end of each interval, t, of one day.

A new technique for changing the composition of the culture medium was introduced. Growth in the complete nutrient medium was maintained until growth rate, population density, and photosynthetic characteristics of the harvested cells were constant over a period of several days. Thereupon a new bottle of culture medium with a changed (or zero) concentration of some one component (*e.g.*, iron) was inserted. As growth continued the culture medium in the chamber changed (was diluted) in respect to the varied component. Population density was held constant, and growth expressed itself only in volume of fresh medium added each day. The actual concentration of the varied component at any time could therefore be calculated. An assumption required is that the distribution of the component between cells and fluid was constant and independent of the concentration in the fluid. This is probably not entirely valid and in this respect the quantitative estimation of the actual concentrations is rendered uncertain for large changes. However, the interpretation of the results of a change in concentration or deficiency of any component is in no way affected.

Photosynthetic characteristics were studied manometrically by the Warburg technique at a temperature of 25° C. as previously described (**7**). Cells were immersed in Knop's solution (pH 4.5) saturated with 4% carbon dioxide and the rate of oxygen evolution calculated on the basis of an assimilatory quotient of – 0.90. Cell volumes were determined on duplicate aliquots by centrifuging in Van Allen thrombocytocrit tubes as previously described (**6**). Rates of photosynthesis could then be expressed in cmm. O_2/hr./cmm. cells. The rate of photosynthesis measured under conditions of light and carbon dioxide saturation is herein described as the *capacity for photosynthesis.*

Results

Comparison of population densities of 0.5 cmm. to 1.5 cmm. cells/cc. has revealed no significant differences in the subsequent photosynthetic behavior of the cells. At the higher population densities the growth rate is somewhat lower, however, a result of increased mutual shading and slightly lower effective light intensity. For subsequent work, therefore, a density of 0.5 cmm. to 0.7 cmm. cells/cc. has been adopted as a convenient working range.

Effects of major salt concentration

Data of an experiment designed to find the effects of changing the major salt concentration by the dilution technique described above are presented (table I). A culture was grown for a period of six days on Knop's solution

TABLE I

EFFECTS OF THE MAJOR SALT CONCENTRATION

DAY	RELATIVE CONCENTRATION AT END OF DAY	GROWTH* RATE	CAPACITY FOR† PHOTOSYNTHESIS	pH OF SUSPENSION
2–6	1.0	0.47	47	5.9
	Insert 2X concentration of major salts			
7	1.65	0.45	—	—
8	1.88	0.46	—	—
9	1.95	0.43	46	—
10	1.98	0.44	47	5.7
	Insert zero concentration of major salts			
11	0.68	0.46	—	5.9
12	0.23	0.47	46	—
13	0.089	0.43	47	7.0
14	0.046	0.28	19	7.0

* $\left(\log \frac{N}{N_0}\right)$ 24 hrs.

† emm. O_2/hr./emm. cells.

at its usual concentration. At the end of the sixth day a new bottle of medium was inserted containing twice the usual concentration of the major salts. From the amount of daily inoculum and daily harvest the relative concentration calculated for the end of each day is expressed in the second column. By the tenth day the major salt concentration had nearly doubled without appreciable effect on growth rate or the capacity of the cells for

TABLE II

EFFECTS OF DEPLETION OF IRON

DAY	Fe CONCENTRATION AT END OF DAY	GROWTH RATE	CAPACITY FOR PHOTOSYNTHESIS	CHLOROPHYLL CONCENTRATION*
1	Insert Knop's containing zero Iron; original concentration had been 13.3×10^{-5} M			
7	0.049×10^{-5}	0.41	42	9.6
8	0.020	0.39	43	—
9	0.0097	0.32	37	9.7
10	0.0044	—	—	—
11	0.0021	0.34	38	8.2
12	0.0010	0.31	33	—
13	0.00049	0.31	23	6.8
14	0.00025	0.30	18	—
	Insert Knop's containing 0.133×10^{-5} M Iron			
15	0.048	—	—	—
16	0.096	0.36	43	—
17	0.128	0.41	42	9.1

* In arbitrary units/emm. cells (5).

photosynthesis. A bottle of medium was then inserted containing zero concentration of the major salts. Continued dilution of the major salt concentration finally gave rise to a reduced rate of growth and capacity for photosynthesis. In respect to these characteristics, however, it is apparent that the cells were insensitive to the major salt concentration over approximately a twenty-fold range. In the light of earlier work by MYERS (4), the development of the relatively high pH of 7.0 probably indicates a complete removal of nitrate from the medium on days 13 and 14 of the experiment.

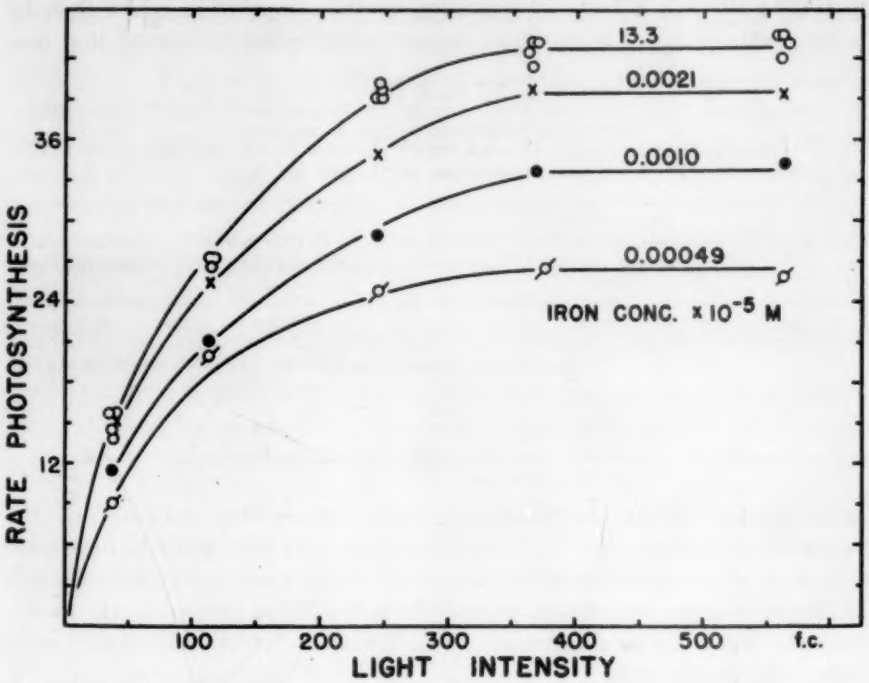

FIG. 1. Light intensity curves of photosynthesis for cells harvested on days 1, 11, 12, and 13 of the experiment described (table II). Values on the curves represent calculated iron concentrations at the time of harvesting. Rate = cmm. O_2/hr./cmm. cells measured in Knop's + 4.4% CO_2.

EFFECTS OF IRON CONCENTRATION

Results of the dilution technique applied to the study of iron concentration are given (table II). Growth rate, capacity for photosynthesis, and chlorophyll concentration were but slightly affected during the 270-fold drop in iron concentration over a six-day period. Further dilution of iron was accompanied by a decrease in all three of the cellular characteristics examined. Effects of iron depletion are shown in terms of the light intensity curves of photosynthesis for cells harvested at days 1, 11, 12, and 13 and labelled with the calculated iron concentrations prevailing at the time of harvesting (fig. 1). Any one of the lower curves can be made

to fit approximately the upper curve by multiplying all points by a single factor. No apparent differential effects of iron concentration on the "photochemical" and "dark" processes are detectable in this experiment.

Effects of microelement concentrations

With the usual iron concentration of 13.3×10^{-5} M it was impossible to demonstrate any effects of depletion of the A_5 or B_6 microelement solutions by the dilution technique. The iron concentration was thereupon reduced to 0.133×10^{-5} M. Effects of depletion of the A_5 solution could then be noted (table III). It is apparent (as previously noted by others) that iron

TABLE III

Effects of depletion of the A_5 solution
(Original concentration = 1.0 ml./liter; contains B, Cu, Mn, Mo, Zn; Iron concentration 0.133×10^{-5} M)

Day	Fraction of orig. concentration at end of day	Growth rate	Capacity for photosynthesis	Chlorophyll concentration
2–5	1.0	0.45	47	9.3
Insert Medium Containing Zero A_5				
6	0.35	0.45	47	—
7	0.13	0.43	44	—
8	0.078	0.22	11	5.3

salts without special purification contain appreciable amounts of the requisite microelements. No further attempts have been made to determine critical levels or effects of deficiency of any of the individual microelements of the A_5 solution since this is beyond the scope of the present work. Even with the lowered iron concentration, no effects of depletion of the B_6 solution could be observed.

Effects of hydrogen ion concentration

The Knop's solution used for growth throughout these experiments was initially adjusted to pH 5.0. This gave a pH of about 5.8 in the culture suspension. Changes in initial pH such that the culture suspension was maintained at pH 5.2 or pH 6.7 revealed no significant effects on the photosynthetic behavior of the harvested cells. However, at higher pH iron slowly precipitated despite the added citrate, and at lower pH the suspensions foamed badly and the algae tended to deposit on the upper walls of the chamber. A pH of culture of about 5.8 seemed to be an optimum for practical operation.

Effects of concentration of carbon dioxide

Comparative data on the effects of provision of air and 4.4% carbon dioxide in air on the subsequent photosynthetic behavior of the cells are

TABLE IV

EFFECTS OF CARBON DIOXIDE CONCENTRATION

CARBON DIOXIDE PROVISION	GROWTH RATE	CAPACITY FOR PHOTOSYNTHESIS
4.4%	0.45	43
Air	0.26	34

shown (table IV and fig. 2). The actual concentrations of carbon dioxide available to cells in the growth chambers is not known. Changes of from 2% to 5% carbon dioxide in the influent gas mixture have been found to be without effect on the growth rate of subsequent photosynthetic activity of the cells. It is therefore assumed that aeration with 4.4% carbon dioxide provides a saturating concentration for photosynthesis. In a culture aerated with air, however, the calculated rate of carbon dioxide utilization approaches the rate of provision in the influent air. The effective concentration in the culture must be considerably less than the initial 0.03% in the influent air. This is borne out by the observation that in air growth rate is dependent on rate of aeration (carefully controlled in the above experiment) while in 4.4% carbon dioxide growth rate is independent of aeration rate.

It has already been shown (7) that at 25 fc growth rate is proportional to light intensity and therefore probably determined by rate of photosyn-

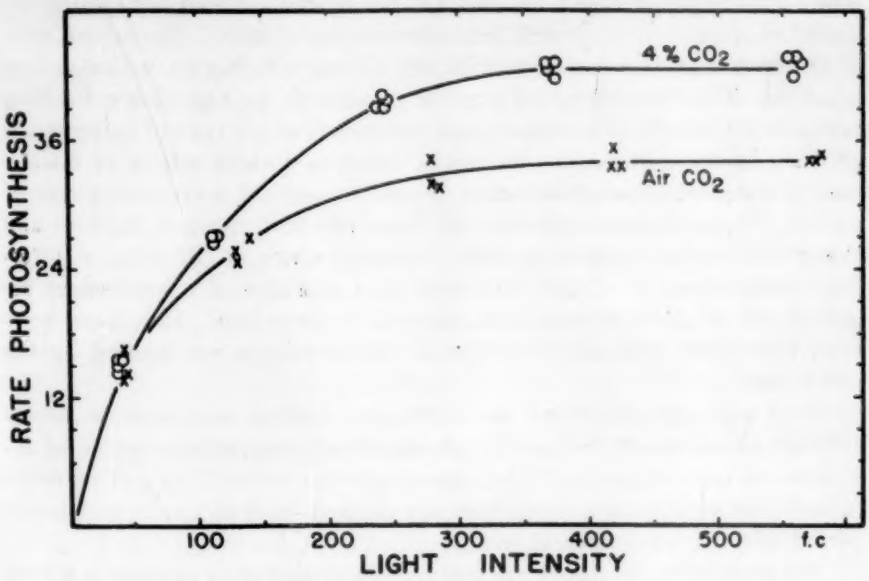

FIG. 2. Light intensity curves of photosynthesis for cells grown at different concentrations of carbon dioxide. Values on the curves indicate carbon dioxide composition of the influent air of the growth chambers. Rate = cmm. O_2/hr./cmm. cells measured in Knop's + 4.4% CO_2.

thesis. EMERSON and GREEN (**2**) have shown that in *C. pyrenoidosa* rate of photosynthesis is independent of carbon dioxide concentration down to less than 0.05%. On these grounds also one may predict a limiting rate of provision of carbon dioxide on aeration with air. The problem of rate of provision of carbon dioxide has been discussed previously (**4**).

Limited provision of carbon dioxide during growth gives rise to cells with a lowered capacity for photosynthesis at high light intensities (Blackman rate) as indicated (fig. 2). On the other hand, limiting carbon dioxide has no effect on the subsequent rates of photosynthesis at low light intensity (photochemical rate).

Further work on effects of limited carbon dioxide provision had been planned. Unfortunately, cells grown under these conditions tend to cling to the sides of the chamber so that further work along this line has been abandoned due to the technical difficulties involved.

Discussion

The techniques described above offer a new approach to the general problem of evaluating the critical concentration of any substance required in the nutrition of a microorganism. Comparison with the conventional method is illustrated by consideration of the work of HOPKINS and WANN (**3**) on the iron requirement for Chlorella. By addition of graded amounts of iron they were able to determine: (1) the minimum concentration at which growth would take place and (2) the amount of iron per culture required to allow development of a maximum crop of cells. The second type of determination is a rather general one although it merely indicates how great the initial concentration must be to prevent its becoming a limiting factor in the growth of a culture. In contrast to either type of information afforded by this method, the technique introduced above allows an estimation of the minimum concentration needed to support a maximum rate of growth. Quantitative comparison of these data with those of Hopkins and Wann is impossible since they used a different strain of Chlorella, a different concentration of citrate, and were then unaware of microelement requirements which must have been provided in their iron. It is clear, however, that quite different physiological characteristics are studied by the two methods.

It is also apparent that the continuous-culture technique is neatly adapted to studies of the specific effects of nutrient deficiencies or of inhibitors on microorganisms. Any given nutrient or inhibitor can be maintained continuously in a culture at any desired level and cells withdrawn periodically for physiological study.

The work herein reported was undertaken primarily to continue a survey of the effects of various culture conditions on photosynthetic characteristics of Chlorella. It has been found, in regard to the composition of the nutrient medium, that the concentrations originally chosen more or less arbitrarily give cells with optimum growth rate and capacity for photosynthesis. Small

changes in these concentrations, which might be induced by changes in other conditions, are without appreciable effects.

Summary

1. *Chlorella pyrenoidosa* was grown in a continuous-culture apparatus. Composition of the nutrient medium, carbon dioxide concentration, and population density were studied for their effects on growth and subsequent phytosynthetic behavior.

2. Growth and photosynthetic behavior are relatively insensitive to large changes in the composition of the medium. The major salt concentration may be varied about 20-fold, iron 270-fold, A_5 microelement concentration 3-fold, hydrogen ion 30-fold, all without effect.

3. Limited provision of carbon dioxide during growth yields cells with a lowered capacity for photosynthesis.

4. The data presented demonstrate the usefulness of the continuous-culture technique in studies on nutrient deficiencies in microorganisms.

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A FURTHER INVESTIGATION OF THE REPLACEMENT OF BORON BY INDOLEACETIC ACID[1]

ROBERT MACVICAR AND W. E. TOTTINGHAM

Received May 13, 1946

It has been reported (**2**) that low concentrations of indoleacetic acid in the nutrient solutions supplied to cotton seedlings will partially replace boron for growth in cultures deficient in this element. This finding constitutes a fundamental advance in the elucidation of the role of boron in the metabolism of the plant. The further implication of auxins in respiration (**1**) and growth indicates that boron might function through some relationship with these enzyme systems. It was believed desirable, therefore, to attempt to confirm this work and extend it to other and more suitable experimental plants.

Experimental data

Plants were grown in washed silica sand in varnished clay pots. Seedlings of suitable size, germinated in either sand or soil, were transplanted into sand which had been thoroughly leached with distilled water. The following experimental plants were used: cotton, unknown variety; sunflower, var. Russian Mammoth; tomato, var. Early Baltimore; tobacco, var. Maryland Mammoth; and soybean, var. Manchu no. 3. Four replications of each treatment were made with the exception of the series harvested on June 21, 1941. In this case only three replications were employed.

The composition of the nutrient solutions was as follows:

KH_2PO_4	0.0018 M
K_2HPO_4	0.0012 M
$Ca(NO_3)_2$	0.0042 M
$CaCl_2$	0.0011 M
$Mg(NO_3)_2$	0.0021 M
$MgSO_4$	0.0011 M
NH_4NO_3	0.0042 M

During periods of low light intensity ammonia nitrate was omitted from the solution. Minor elements were included in the concentrations recommended by HOAGLAND (**3**). In series 1 the solution was supplied twice daily by a manually operated air pressure type subirrigation system. In series 2, approximately 500 ml. of nutrient solution was supplied manually each day. Unbalanced solution was removed by weekly leaching with distilled water. The indoleacetic acid was supplied in the nutrient solution at a level of 1 p.p.m. when the subirrigation culture was employed. In the latter series it was added separately and cumulatively each day in such amounts as to pro-

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

duce a concentration of 0.2 p.p.m. in the solution, assuming saturation of the sand.

Normal greenhouse conditions prevailed. During periods of high light intensity, plants were kept in whitewashed houses having intensities of approximately 1000 foot candles. This was similar to the intensity employed by EATON (**2**). Summer temperatures varied between 65° to 90° F. during the day and 60° to 80° F. during the night. Winter temperatures were 65° to 70° F. and 60° to 65° F., respectively. Photoperiods were normal during the summer months and were lengthened to 16 hours by means of incandescent lamps during the winter.

TABLE I

SERIES 1. EFFECT OF INDOLEACETIC ACID ON GROWTH OF PLANTS IN THE PRESENCE AND ABSENCE OF BORON IN THE NUTRIENT MEDIUM

PLANT, TREATMENT, AND DATE OF SAMPLING	DAYS OF IAA TREATMENT[2]	WET WEIGHT OF TISSUE PER PLANT		DRY WEIGHT OF TISSUE PER PLANT		DRY MATTER		TOP / ROOT
		TOP	ROOT	TOP	ROOT	TOP	ROOT	
		gm.	*gm.*	*gm.*	*gm.*	%	%	
June 26, 1941								
Sunflower, CN[1]	.....	41.7	15.9	3.73	0.80	8.9	5.0	4.6
" CN–B	.....	15.3	6.2	1.61	0.40	9.5	6.5	4.0
" CN + IAA[1]	15	40.0	16.9	3.17	0.95	7.9	5.6	3.5
" CN + IAA–B	15	18.2	6.7	1.65	0.44	9.1	6.6	3.8
Cotton, CN	.....	21.8	8.9	2.27	0.52	9.6	5.9	4.4
" CN–B	.....	8.1	3.8	1.08	0.32	10.3	8.4	3.4
" CN + IAA	15	19.4	6.95	2.13	0.36	11.0	5.2	5.9
" CN + IAA–B	15	12.3	8.3	1.14	0.47	9.3	5.6	2.4
Soybean, CN	.....	28.6	18.9	4.26	1.27	14.4	6.7	3.0
" CN–B	.....	18.9	15.8	2.83	1.05	15.0	6.0	3.8
" CN + IAA	15	32.6	20.9	4.71	1.35	14.4	6.5	2.9
" CN + IAA–B	15	15.8	13.2	2.72	0.95	15.5	7.2	2.9
Dec. 13, 1941								
Tobacco, CN	.....	253.6	32.8	20.80	2.68	9.2	8.2	7.8
" CN–B	.....	101.0	15.4	9.99	1.52	10.9	9.9	6.6
" CN + IAA	65	252.7	41.7	21.98	3.61	10.4	8.7	8.3
" CN + IAA–B	65	167.7	21.6	14.42	1.86	7.5	8.6	7.8

[1] CN, complete nutrient; IAA, indoleacetic acid.
[2] Concentration 1.0 p.p.m. in nutrient solution.

Under the conditions used, boron deficiency symptoms appeared in those plants not receiving this element without regard to the presence or absence of indoleacetic acid. No compensatory action was noted either in the time of onset or degree of intensity of deficiency symptoms. This observation was substantiated by anatomical observations of the stems.[2] Examination of sections of stem taken at the fourth internode from the stem tip showed the necrotic areas typical of boron deficiency in the region of the first formed xylem and the surrounding xylem parenchyma cells regardless of the presence of indoleacetic acid in the culture.

Examination of the data showed that in only one instance was there any

[2] Appreciation is expressed to B. ESTHER STRUCKMEYER, Department of Horticulture, University of Wisconsin, who prepared and examined these sections.

TABLE II

SERIES 2. EFFECT OF INDOLEACETIC ACID ON GROWTH OF PLANTS IN THE PRESENCE AND ABSENCE OF BORON IN THE NUTRIENT MEDIUM

PLANT, TREATMENT AND DATE OF SAMPLING	DAYS OF IAA TREATMENT[2]	WET WEIGHT OF TISSUE PER PLANT			DRY WEIGHT OF TISSUE PER PLANT			DRY MATTER			TOP / ROOT RATIO
		LEAF	STEM	ROOT	LEAF	STEM	ROOT	LEAF	STEM	ROOT	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%	%	%	
October 17, 1941											
Sunflower, CN	.....	15.9	30.9	17.3	2.26	2.62	1.63	14.2	8.5	9.4	3.3
" CN–B	.....	7.5	13.6	6.2	0.81	0.91	0.49	10.8	6.7	7.9	3.5
" CN + IAA[1]	40	14.5	29.9	14.0	2.04	2.82	1.32	14.1	9.5	9.4	2.8
" CN + IAA–B	40	7.5	18.6	9.7	1.26	1.22	0.75	16.8	6.6	7.7	3.2
January 16, 1942											
Tomato, CN	.....	40.7	31.7	20.9	4.59	3.79	1.72	11.2	8.6	8.2	4.9
" CN–B	.....	30.1	21.8	13.1	2.87	1.99	1.25	9.6	9.1	9.5	4.9
" CN + IAA	30	37.9	31.9	22.1	4.00	3.23	1.67	10.5	10.1	7.6	4.2
" CN + IAA–B	30	31.9	21.2	11.2	2.90	1.79	0.96	11.0	11.8	8.6	4.8
Sunflower, CN	.....	14.2	38.9	9.6	1.63	2.69	0.68	11.5	6.9	7.1	6.4
" CN–B	.....	8.9	12.3	5.4	0.85	0.73	0.24	10.5	5.9	4.5	6.6
" CN + IAA	30	16.0	37.1	13.2	1.88	3.94	0.95	8.5	9.1	7.2	6.1
" CN + IAA–B	30	7.5	13.1	5.3	0.79	0.81	0.23	10.5	5.8	4.4	6.5

[1] CN, complete nutrient; IAA, Indoleacetic acid.
[2] Concentration 1.0 p.p.m. in solution in October 17, 1941 sample; added daily and cumulatively at 0.2 p.p.m. in January 16, 1942 sample.

significant increase in the dry weight of boron deficient plants receiving indoleacetic acid (tables I and II). In this instance, the tobacco harvested on December 19, 1941, was significantly higher when indoleacetic acid was supplied. It had been noted, however, that the symptoms of boron deficiency appeared somewhat earlier in those plants receiving indoleacetic acid and more secondary tissue was produced from developing axillary buds. Renewed growth in such circumstances is frequently observed in tobacco that has become severely deficient (**5**). It is believed that the somewhat greater mass of tissue produced by plants receiving indoleacetic acid may have been principally due to the earlier appearance of initial symptoms. No significant correlations of moisture content or root-top ratios to the presence or absence of either boron or indoleactic acid were found.

Discussion

It is recognized that the technique employed in this study differed in several major respects from that of EATON (**2**). He used unaerated solution culture and produced symptoms of boron deficiency at a much earlier stage of growth than is possible under normal conditions in sand cultures. Whether any protective action of indoleacetic acid in conditions producing boron deficiency is more efficient in the seedling than in the more mature plant is, as yet, undetermined. The concentration of indoleacetic acid, moreover, was higher in the sand culture experiments.

It may be that other environmental factors are involved in the differences of results obtained. Eaton noted that the protective action of indoleacetic acid was largely nullified by high light intensities (**2**), and it may be that this effect is more pronounced in the older plants. It has also been observed that the severity of boron deficiency may be accentuated by high levels of calcium (**6**). The solution employed in this study contained higher levels of calcium ion than did that used by Eaton in his study. HOAGLAND and SNYDER (**4**) have suggested that the degree of aeration of nutrient solutions may be related to the severity of boron deficiency. Suitable conditions for repeating these experiments under more rigidly controlled conditions of light intensity and other environmental factors are not available to us. Elucidation of a possible relation between boron and the auxins, however, might well provide valuable insight into the metabolic function of both.

Examination of the data indicates, however, that under the conditions employed any appreciable replacement of boron by indoleacetic acid in growing plants was absent. Whether or not it occurs under more suitable and limited environmental conditions is still unknown.

Summary

No significant alleviation of boron deficiency by indoleacetic acid was noted in sand culture under normal greenhouse conditions with tomato, cotton, sunflower, soybean, and tobacco plants.

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GERMINATION OF *ASYSTASIA GANGETICA* L. SEED WITH SPECIAL REFERENCE TO THE EFFECT OF AGE ON THE TEMPERATURE REQUIREMENT FOR GERMINATION[1]

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Introduction

Freshly harvested seed of *Asystasia gangetica* L., an ornamental shrub, does not germinate well under ordinary conditions. This seed normally requires a storage period of several months at room temperature before it germinates. The purpose of this investigation is to determine the requirements for germination of the seed at different ages.

The literature on the use of alternating temperatures to break the dormancy of seeds is voluminous, and no attempt will be made here to refer to all of the reported cases. HARRINGTON (**2**) extensively studied the use of alternating temperatures to increase the germination of seeds of grasses and garden crops. MORINAGA (**3**) increased the germination of Bermuda grass seed with alternating temperatures. TOOLE (**4**) also used alternating temperatures to improve the germination of other grass seeds. It has been the experience of the author that the germination of seeds of some of the grass species grown on Hawaiian ranges is materially improved by the use of alternating temperatures (**1**).

Methods

Seeds used in the investigation reported herein were harvested at maturity from Asystasia plants grown on the Hawaii Agricultural Experiment Station grounds. Curing was effected by storing the seeds at room temperature conditions until they were used for germination tests.

Germination tests were conducted in Petri dishes using filter paper moistened with distilled water. Ovens and cold storage chambers were used for temperature variations. Temperatures employed consisted of: 6.6°–10.2°, 15.0°, 20.8°–28.0° (room temperature), 29.9°–33.2°, and 36.0° C. and combinations of these for the alternating temperatures. For temperature alternations, the Petri dishes with the seeds therein were transferred from one temperature to another at regular intervals. The schedule of daily alternating temperatures listed in table I was followed, and except in some unavoidable cases, the daily 8-hour period extended from 8 A.M. to 4 P.M. and the 16-hour period from 4 P.M. to 8 A.M. the following day. The number of seeds used for the germination tests varied from 25 to 100 seeds per treatment. In a few cases where the seed supply was sufficient, replicated tests were conducted.

[1] Published with the approval of the Director as Technical Paper no. 155 of the Hawaii Agricultural Experiment Station.

Results

Results of many tests to increase the germination of freshly harvested seed of Asystasia indicated that temperature has a marked influence on germination and that the alternating temperature treatment is the most effective. At continuous temperatures the dormant seed does not germinate as well as at alternating temperatures. Whatever temperature treatment is necessary to break the dormancy, a minimum temperature of approximately 20° C. must be provided for actual germination.

Removal of the seedcoat of normally dormant seed produces a germination of approximately 75% at room temperature, whereas the intact seed produces no germination or at best a very low germination. The maximum germination produced by this treatment, however, is not so high as the

TABLE I

SCHEDULE OF DAILY ALTERNATING TEMPERATURE TREATMENTS

TEMPERATURE ALTERNATION	PERIOD AT LOWER TEMPERATURE	PERIOD AT HIGHER TEMPERATURE
°C.	*hours*	*hours*
6.6–10.2 to 15.0	8	16
6.6–10.2 to 20.8–28.0 (room temperature)	8	16
6.6–10.2 to 29.9–33.2	8	16
6.6–10.2 to 36.0	8	16
15.0 to 20.8–28.0	8	16
15.0 to 29.9–33.2	8	16
15.0 to 36.0	8	16
20.8–28.0 to 29.9–33.2	16	8
20.8–28.0 to 36.0	16	8
29.9–33.2 to 36.0	16	8

germination (about 90% and over) obtained by the use of optimum alternating temperatures.

After performing a number of germination tests with seeds harvested at different times, it became apparent that seeds of different ages respond differently to the various temperature treatments. Attempts were then made to determine the temperature requirement for maximum germination at different ages (since harvest). A complete tabulation of all of the data is not included in this report, but a compilation of some of the results obtained in these trials is recorded in table II and a résumé of all tests conducted on different lots of seed harvested at various times is presented in table III which shows the temperature requirement for germination of seed of different age groups.

From tables II and III it is seen that for germination after one week of curing, the fresh seed requires a severe temperature treatment, and as the seed ages, this treatment becomes less and less severe until finally the seed requires no special treatment and germinates at room temperature (20.8°–28.0° C.). When the seed is only two to seven days old, it requires an

TABLE II

EFFECT OF TEMPERATURE VARIABLES ON THE GERMINATION OF *Asystasia gangetica* L. SEED OF DIFFERENT AGES

AGE OF SEED SINCE HARVEST*	TEMPERATURE TREATMENT		NO. OF SEEDS USED	GERMINATION IN TWO WEEKS
days		°C.		%
	Alternating	6.6–10.2 to 20.8–28.0 (room temperature)	100	51.7
	"	6.6–10.2 to 29.9–33.2	100	67.2
	"	6.6–10.2 to 36.0	100	51.7
	"	15.0 to 20.8–28.0	100	86.0
7	"	15.0 to 29.9–33.2	100	88.0
	"	15.0 to 36.0	100	63.0
	Continuous	20.8–28.0	100	1.7
	Alternating	20.8–28.0 to 29.9–33.2	100	84.7
	"	20.8–28.0 to 36.0	100	93.2
	Alternating	6.6–10.2 to 29.9–33.2	75	91.9
	"	6.6–10.2 to 36.0	75	100.0
	Continuous	20.8–28.0	75	0.0
14	Alternating	20.8–28.0 to 29.9–33.2	75	30.0
	"	20.8–28.0 to 36.0	75	60.0
	Continuous	29.9–33.2	75	0.0
	"	36.0	75	26.1†
	Alternating	6.6–10.2 to 29.9–33.2	100	97.4
	"	6.6–10.2 to 36.0	100	68.4
	"	15.0 to 20.8–28.0	100	93.0
20	"	15.0 to 29.9–33.2	100	94.0
	"	15.0 to 36.0	100	76.0
	"	20.8–28.0 to 29.9–33.2	100	44.7
	"	20.8–28.0 to 36.0	100	50.0
	Alternating	6.6–10.2 to 20.8–28.0	100	94.7
	"	6 6–10.2 to 29.9–33.2	100	90.0
	"	6.6–10.2 to 36.0	100	84.2
28	Continuous	20.8–28.0	100	5.2
	Alternating	20.8–28.0 to 29.9–33.2	100	79.7
	"	20.8–28.0 to 36.0	100	87.5
	Continuous	29.9–33.2	100	12.1
	"	36.0	100	29.3†
	Alternating	15.0 to 20.8–28.0	100	99.0
58	"	20.8–28.0 to 29.9–33.2	100	56.0
	"	20.8–28.0 to 36.0	100	35.0
	Alternating	15.0 to 20.8–28.0	50	87.0
84	Continuous	20.8–28.0	50	8.0
	Alternating	20.8–28.0 to 29.9–33.2	50	84.0
	"	20.8–28.0 to 36.0	50	92.0
	Continuous	20.8–28.0	25	16.7
	Alternating	20.8–28.0 to 29.9–33.2	25	91.7
112	"	20.8–28.0 to 36.0	25	89.0
	Continuous	29.9–33.2	25	0.0
	"	36.0	25	41.7†
	Alternating	15.0 to 29.9–33.2	25	91.7
	Continuous	20.8–28.0	25	96.8
140	Alternating	20.8–28.0 to 29.9–33.2	25	100.0
	Continuous	29.9–33.2	25	41.7
	"	36.0	25	50.0†

* Seed cured and stored at room temperature conditions.

† Even though germination at 36.0° C. was superior to that at 29.9°–33.2° C., it is probable that the higher temperature is excessive for germination of the seeds continuously exposed to it because injury to the seeds was apparent at this temperature.

alternation between room temperature and 36.0° C. for germination. When the seed is 8 to 14 days old, it requires a more drastic treatment—an alternation between the lowest and the highest temperatures used. This difference in the germination requirement in the two age groups is probably due to the fact that in one case the seed—especially the seedcoat—is not fully cured, thus requiring a less severe treatment, whereas in the other, the seed is fully cured, thus requiring more extreme temperatures in the alternation. When the seed is older than 14 days, it requires less and less drastic treatment for germination as the seed ages. Finally when the seed is 135 days old and older it germinates normally at room temperature.

TABLE III

THE OPTIMUM TEMPERATURE CONDITIONS FOR MAXIMUM GERMINATION AT THE VARIOUS AGES OF THE SEED OF *Asystasia gangetica* L.

AGE OF SEED SINCE HARVEST*	TEMPERATURE REQUIRED FOR MAXIMUM GERMINATION	
days		°C.
2 to 7	Alternating	20.8–28.0 (room temperature) to 36.0
8 to 14	"	6.6–10.2 to 36.0
15 to 26	"	6.6–10.2 to 29.9–33.2
27 to 36	"	6.6–10.2 to 20.8–28.0
37 to 82	"	15.0 to 20.8–28.0
83 to 88	"	20.8–28.0 to 36.0
89 to 134	"	20.8–28.0 to 29.9–33.2
135 to 168	Continuous	20.8–28.0 or alternating 20.8–28.0 to 29.9–33.2

* Seed cured and stored at room temperature conditions.

Discussion

Since removal of the seedcoat promotes germination at room temperature, though not to a degree of alternating temperatures, it seems that the cause of dormancy in the freshly harvested seed of Asystasia mainly lies in the nature of the seedcoat. The difference in germination percentage resulting from the removal of seedcoat and the use of alternating temperatures is probably due to the more favorable influence of the alternating temperatures on the respiration process of the seed. The seedcoat readily allows passage of water, but it probably restricts the passage of gases involved in respiration. Removal of the seedcoat might eliminate this obstruction to gas movement.

A higher germination with alternating temperatures than with continuous temperatures was obtained in dormant Asystasia seed (table II). HARRINGTON (**2**) states that the beneficial effect of alternating temperatures on germination is due not to the specific effect of the extreme temperatures of the alternation or to the mean temperature of the alternation but to the changes in the temperatures. Since removing the seedcoat allows for germination, it seems probable that the alternating temperatures render the intact seedcoat more permeable to essential materials required for germination.

Since the severity in the temperature alternation necessary for germination decreases with the increase in age of the seed (tables II and III), it seems that normally the change in permeability comes about through the aging (after 7 days) of the seedcoat. Perhaps simultaneously the afterripening process proceeds as the seed ages. Thus with the aging of the seed, the severity of the temperature treatment required for germination decreases until finally when the seedcoat is no longer an obstruction to essential materials the seed germinates under ordinary conditions.

Summary

1. Freshly harvested seed of *Asystasia gangetica* L. does not germinate well at room temperature.

2. After a period of approximately 135 days in ordinary storage, it germinates readily at room temperature.

3. Seeds between the ages of 8 and 134 days require an alternation of temperatures for germination, the severity of which is related to the age of the seed—the younger the seed the more severe the required treatment and *vice versa*.

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THE EFFECT OF ULTRAVIOLET LIGHT ON SUBSEQUENT RIPENING OF THE FRUIT OF THE TOMATO (*LYCOPERSICON ESCULENTUM*)

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Introduction

While conducting physiological studies on the development of color in Florida oranges, it was observed that frequently midseason fruits developed a rich orange color when they were growing on the northwest side of the tree and a pale yellow color when growing on the southeast side. It appeared that this difference in color of the fruits was in some way related to the unequal exposure to sunlight and quite possibly the result of different amounts of ultraviolet light received. A few tests were therefore made to determine the effects of ultraviolet irradiation on pigment development in fruits in the hope that the information obtained might contribute to a better understanding of color development in citrus fruits. Preliminary studies indicated that ultraviolet irradiation of harvested, mature-green oranges retarded the rate of degreening of these fruits but did not in any way effect the yellow pigments. The conditions in the laboratory in these experiments were not quite comparable to those existing in the grove because oranges do not show an increase in carotenoid pigments after removal from the tree. However, it was not practicable to subject the oranges to ultraviolet irradiation while they were still attached to the tree. The only alternative was to select a fruit which had been shown to increase in carotenoid pigments after removal from the parent plant. It was for this reason that subsequent experiments were conducted with fruits of the tomato (*Lycopersicon esculentum*).

Materials and methods

Mature green tomatoes were sorted into lots of 10 to 20 each, the fruits in each lot being comparable in regard to size and degree of maturity.[2] One lot (original sample) was analyzed immediately for chlorophyll and carotenoid pigments. Other lots were given ultraviolet irradiation by means of a "Sterilamp"[3] placed 18 inches above the fruits. The position of the

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[2] The term "mature green" is applied to green tomatoes that will ripen after removal from the vine. They consist of fruits that have advanced from the dark green to the light green stage. However, since these changes are progressive and since the fruits are selected entirely by appearance, the term actually represents a range rather than a specific stage of maturity. In making up the experimental lots an attempt was made to select fruits of a uniform color.

[3] According to Westinghouse Company, manufacturers of the "Sterilamp," energy is emitted by this lamp in wave lengths ranging from 2537 to 5780 Angstrom units, with the lower wave lengths representing 83.03% of the total. The intensity at a distance of

tomatoes was reversed during the period of exposure in order to permit both stem-end and blossom-end to receive an equal amount of irradiation. A stream of air directed over the irradiated fruits prevented the temperature from rising above that of the control lot. Following the irradiation the tomatoes were held in the 70° F. room until the majority of the control fruits were fairly well colored. In most cases the fruits were given a preliminary treatment with ethylene gas, in order to shorten the time required for ripening.

Total chlorophyll and total carotenoid pigments were determined by a method reported previously (**2, 3**). Essentially, the method consisted of extraction of the fresh pulp with acetone, transferring the extract to petroleum ether, and purifying. The chlorophyll was separated by saponification and determined colorimetrically by means of a Clifford photometer. The total carotenoids (remaining in the petroleum ether) were likewise determined colorimetrically. Total acidity was determined by extracting 100 grams of the pulp with boiled distilled gater, filtering, and titrating the filtrate with standard NaOH. Details of this method have been published elsewhere (**1**).

Results

The results obtained in the first experiment are presented in table I. It will be noted that at the end of the ripening period all of the fruits in lot 2 (no irradiation) were either pink or red. Lot 3 (3 hours' irradiation) contained seven pink and no red fruits, while lot 4 (5 hours' irradiation) contained six pink fruits and one red fruit. The others in these two lots were either green or turning. That the exposure to ultraviolet light retarded ripening was evidenced by both the failure to lose the green color and the failure to produce the orange red pigments.

In order to express the data quantitatively the fruits were analyzed for chlorophyll and carotenoid pigments. These data are also presented in table I. The sample taken at the beginning of the experiment (lot 1) contained 2.85 milligrams of chlorophyll and 0.417 mg. of carotenoid pigments per 100 grams of flesh. After the two days' ripening period the control lot contained 0.55 mg. of chlorophyll and 1.662 mg. of total carotenoids per 100 grams. The two irradiated lots had not lost as much chlorophyll nor had they produced as great a quantity of carotenoid pigments as the control lots during the same time. Lot 3 (irradiated 3 hours) contained 1.12 mg. of chlorophyll and 1.125 mg. of total carotenoids. Lot 4 (five hours' irradiation) contained 1.00 mg. of chlorophyll and 1.140 mg. of total carotenoid pigments per 100 grams of flesh. In other words, the control lot had produced at least 45% more carotenoid pigments than had the irradiated lots.

Table II shows the effect of ultraviolet irradiation on the subsequent

18 inches would be equal to 150.19 microwatts per square centimeter. The shortest measurable rays in solar radiation are approximately 2900 Angstrom units. It is thus apparent that the radiations from the Sterilamp were not equivalent to the ultraviolet radiation in sunlight.

TABLE I

EFFECT OF ULTRAVIOLET IRRADIATION ON SUBSEQUENT RIPENING OF TOMATO FRUITS

LOT	PERIOD OF IRRADIATION	NUMBER OF FRUITS IN EACH COLOR GROUP				TOTAL CHLOROPHYLL	TOTAL CAROTENOIDS
		GREEN	TURNING	PINK	RED		
						mg./100 gm.	*mg./100 gm.*
1	0 (orig. sample)	10	0	0	0	2.85	0.417
2*	0 (control)	0	0	5	5	0.55	1.662
3	3 hours	2	1	7	0	1.12	1.125
4	5 hours	3	0	6	1	1.00	1.140

* Record made of lots 2, 3, and 4 after two days in ethylene.

ripening of Rutgers tomatoes. It is significant that there were no fully ripe tomatoes in any lot except the one receiving no irradiation. In fact, there were no pink fruits in any irradiated lot except number 4 (two hours' irradiation), which contained eleven. Some of the fruits in this lot may have been more mature than others when sorted. Except for those in lot 4 none of the irradiated fruits had progressed beyond the "green" or "turning" stage at the end of the ripening period.

Table III shows the results of a second experiment conducted with Rutgers tomatoes in Florida. The original sample contained 2.13 mg. of chlorophyll and 0.225 mg. of total carotenoids per 100 grams of flesh. After two days in ethylene and five days in air at 70° F. the control lot had lost all of its chlorophyll and had produced 5.24 mg. of total carotenoids per 100 grams of flesh. As in previous experiments the fruits in the irradiated lots lost less chlorophyll and produced less carotenoid pigments than did those in the control lots. The values for these two lots expressed as mg. of pigment per 100 grams of flesh are as follows: Lot 3 (two hours' irradiation), 1.19 mg. of chlorophyll and 2.120 mg. of carotenoids; lot 4 (four hours' irradiation), 1.00 mg. of chlorophyll and 0.657 mg. of carotenoids.

TABLE II

EFFECT OF ULTRAVIOLET IRRADIATION ON SUBSEQUENT DEVELOPMENT OF COLOR IN GREEN TOMATOES*

LOT	PERIOD OF IRRADIATION	NUMBER OF FRUITS IN EACH COLOR GROUP			
		GREEN	TURNING	PINK	RED
1	0 (orig. sample)	18	0	0	0
2	0 (control)	7	5	2	4
3	1 hour	8	10	0	0
4	2 hours	2	5	11	0
5	3 hours	9	9	0	0
6	4 hours	14	4	0	0

* Record on all but original sample made after two days in ethylene and five days in air at 70° F.

It seemed desirable at this time to determine whether ripening processes, other than development of color, were affected by the ultraviolet light. Accordingly the pulp of the fruit in the last experiment was analyzed for total acids. The control lot contained 0.540% acid; the lot receiving two hours' irradiation, 0.630%; and the lot which was irradiated four hours contained 0.755%. These data indicate that exposure to ultraviolet light retards the loss of acidity in green tomatoes.

TABLE III

EFFECT OF ULTRAVIOLET IRRADIATION ON TOTAL CHLOROPHYLL, CAROTENOIDS, AND ACIDS IN GREEN TOMATO FRUITS*

LOT	PERIOD OF IRRADIATION	CHLOROPHYLL	CAROTENOIDS	ACID
		mg./100 gm.	*mg./100 gm.*	%
1	0 (orig. sample)	2.13	0.225	.....
2	0 (control)	.....	5.240	0.540
3	2 hours	1.19	2.120	0.630
4	4 hours	1.00	0.657	0.755

* Record on all but original sample made after two days in ethylene and five days in air at 70° F.

Discussion

When a mature green tomato is removed from the vine it will, under satisfactory conditions, lose its green pigment and develop a good red color. The green pigment disintegrates and a considerable quantity of the orange-red pigments is manufactured. Ultraviolet irradiation has been shown to arrest both the destruction of the green and the production of the orange-red pigments. Smith (**4**) has reported that lycopene formation in tomatoes is favored by protection from the light whereas exposure to light is conducive to the development of carotene. Since lycopene constitutes the bulk of the carotenoid pigments in ripe tomatoes, it is apparent in these experiments that ultraviolet irradiation inhibits formation of lycopene.

A maturing orange, while still on the tree, is much like a tomato in that the chlorophyll is decreasing while the carotenoid pigments are increasing. Once an orange is removed from the tree, however, the green pigments disappear, revealing the yellow pigments that have been manufactured up to this point, but there is no increase in carotenoid pigments after the orange has been harvested. This is the reason that the oranges that have been degreened with ethylene usually have a pale yellow and not a deep orange color.

Chromatographic analyses in this laboratory have indicated that while there is a certain amount of carotene in orange rinds, the deep orange color is due to lycopene. This applies to the highly colored oranges of the Mandarin type, to the sweet oranges grown in the northern part of Florida, and even to midseason oranges produced on the northwest side of the trees.

Evidence that ultraviolet light in the sun prevents the formation of pig-

ments in the exposed fruits on the southeast side of the tree is therefore only circumstantial and should not be taken too seriously, but it suggests that this is a field which should be explored for both practical and scientific reasons.

Summary

Exposing mature green fruits of the tomato (*Lycopersicon esculentum*) to the rays of an ultraviolet lamp tended to retard subsequent ripening. The irradiation retarded the loss of chlorophyll, the development of carotenoid pigments, and the reduction of acidity.

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INFLUENCE OF HIGH, MEDIUM, AND LOW SOIL MOISTURE ON GROWTH AND ALKALOID CONTENT OF *CINCHONA LEDGERIANA*

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(WITH THREE FIGURES)

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Introduction

Soil moisture in Cinchona growing regions may fluctuate greatly with seasonal changes in rainfall; prolonged droughts are experienced almost annually. On the other hand, during the peak of the rainy season the same soils may remain at or near saturation for extended periods. For example, at the Toro Negro National Forest in Puerto Rico, where Cinchona is being grown experimentally, the 23-year-average rainfall for two-week periods from January 1 to March 25 was 2.04 inches with frequent periods of drought. In the current year only 0.21 inch fell during January. From August 13 to November 18 rainfall averaged 5.69 inches for two-week periods. The average total annual rainfall for 23 years was 96.37 inches (**4**).

The above extremes in soil moisture may be expected to exert marked influence on the growth and composition of Cinchona. HEINICKE, BOYNTON and REUTHER (**3**) demonstrated that a flooded (poorly aerated) soil produced symptoms in the apple typical of boron deficiency when the soil was not deficient in that element. It was also indicated that lack of oxygen in the soil interfered with the absorption of nitrogen and potassium. WAGER (**6**) showed that avocado plants were more susceptible to disease if grown in a flooded soil than if watered normally.

During the first year from seed, Cinchona plants are primarily herbaceous. It is during this tender stage that soil moisture is most likely to be a critical factor affecting growth. Since the plants are grown in palm-leaf-covered nurseries during this stage it is fairly easy to correct a deficiency of soil water by irrigation, but more difficult to prevent an excess during the rainy season. Cinchona nursery plantings have been observed which were apparently healthy during three-fourths of the year, but with the advent of heavy daily rains during the peak of the rainy season the plants developed symptoms typical of nutrient deficiencies and many succumbed to fungus attack.

The experiment reported here was designed to study the effect of low, medium, and high levels of soil moisture on growth and alkaloid content of young *Cinchona ledgeriana* seedlings under controlled conditions.

Materials and Methods

In March 1946 seedlings of *Cinchona ledgeriana* were transplanted to soil in concrete benches located in three air-conditioned greenhouse chambers, described elsewhere (7). The bench in each chamber was divided into three randomized plots each about 25 inches square. The plots were separated by a double partition of boards spaced 4 inches apart. One hundred and forty-five pounds of a uniform mixture of soil were placed in each plot. The soil consisted of equal parts of clay loam, silica sand, peat moss, and leafmold. The wilting point was 9.5% moisture and the field capacity approximately 36%. The wilting coefficients were determined as the percentage of moisture remaining in the soil at the time of permanent wilting of soybean plants growing in the soil. With this information on soil moisture characteristics it was possible to establish treatments as follows: (1) Low soil moisture ranging between 9 and 13%, (2) medium soil moisture ranging between 18 and 24%, and (3) high soil moisture ranging between 30% and field capacity.

The Cinchona seedlings were selected for uniformity and were about 4 inches high when transplanted. Sixteen seedlings, five inches apart, were planted to each plot.

The temperature in all chambers was maintained at 75° F. during the day and 65° F. at night. In the previous experiment (7) this temperature was found to be the most favorable for growth of *C. ledgeriana*. Relative humidity was maintained as high as possible by continually spraying the floors with water. During the middle of the day relative humidity usually dropped to 60 or 70% but most of the time it ranged between 80 and 95%.

All plots were watered uniformly at regular intervals through March and most of April. On April 25 all plots were watered to field capacity and then allowed to dry gradually until the wilting percentage was approached on June 14. Samples for soil moisture determinations were taken at weekly intervals during the drying period and from these data the approximate rate of water loss in each plot was calculated. Since the weight, volume, wilting point, and field capacity of the soil were the same in each plot it was possible to determine fairly closely the amount of water needed to bring the soil moisture content to the desired levels.

At the time the differential soil moisture treatments were begun on June 21 the soil moisture in all plots was near the wilting point. Different volumes of water were added to the various plots in order to raise the level of soil moisture to the maximum percentage for each treatment range. For example, sufficient water was added to the low-moisture plots to bring the level to 13%, to the medium-moisture plots to bring the level to 24%, and to the high-moisture plots to bring the level to field capacity. The approximate volume of water necessary thereafter for each plot was determined from tables prepared from data obtained as described above. The required volume of water for each plot was applied uniformly over the entire area

with a sprinkling can. At intervals of 7 to 10 days soil plugs ½ inch in diameter were taken from four locations in each plot. Each plug extended to the entire depth of the plot so that the moisture percentage obtained represented a fair average of the volume of soil in which the roots were distributed. After the sample was taken the holes were filled and a new location sampled at the next date. The percentages of soil moisture were determined from the loss in weight of soil dried for 24 hours at 105° C. From the soil moisture data thus obtained it was possible to estimate the approximate time required for a plot to reach the point when water should be needed. Also, maintenance of the desired soil moisture percentages was facilitated by the fact that the rate of water loss by transpiration and evaporation was fairly uniform due to controlled temperature and humidity in the chambers.

The experiment was terminated on January 6, 1947, at which time the soil was washed from the roots. Data on height and fresh and dry weights were obtained and the top-root ratio was calculated. The roots and stems were analyzed for quinine and total alkaloids by the method described by LOUSTALOT and PAGÁN (5). The leaves were analyzed for ash, nitrogen, phosphorus, potassium, calcium, and magnesium by the methods outlined by DROSDOFF and PAINTER (1).

Results

EFFECT OF SOIL MOISTURE ON GROWTH AND ALKALOID CONTENT

Figure 1 shows a weekly record of the soil moisture percentage in the three plots of replication Number 1 at the various sampling dates from June 18, 1946, to January 4, 1947. These data are typical of those from

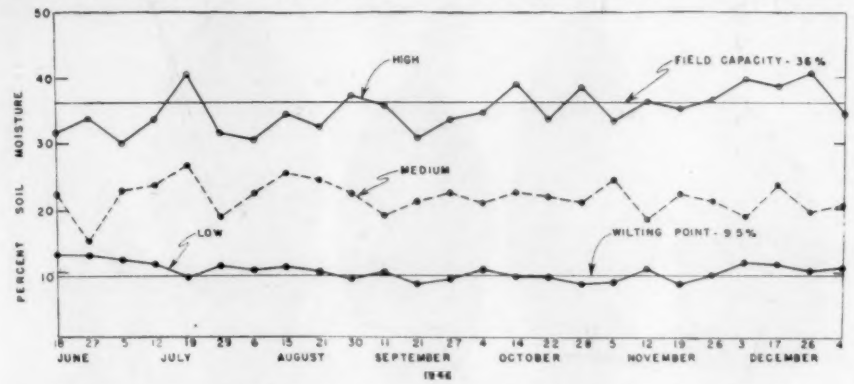

FIG. 1. A typical soil moisture record for plots maintained at high-, medium-, and low-moisture levels. Growth of Cinchona seedlings in these plots is shown in figure 2.

the other two replications. There were some rather wide fluctuations in soil moisture at first, particularly in the medium-soil-moisture plots, but with additional experience it was possible later to control the soil moisture within the designated range. The moisture level that fluctuated the least and consequently was the easiest to control was the low-soil-moisture treat-

FIG. 2. Left to right: typical Cinchona plants grown in plots maintained at high, medium, and low levels of soil moisture. Note crinkling and spotting of tip leaves of plant grown in high soil moisture on the left (see also figure 3).

ment. Only sufficient water was supplied in this treatment to keep the soil moisture about 1 to 3% above the wilting percentage of 9.5. Although on several occasions the soil moisture dropped below the wilting percentage, there was no pronounced evidence of wilting. This was probably due to the fact that the temperature in the chambers was cool and the humidity

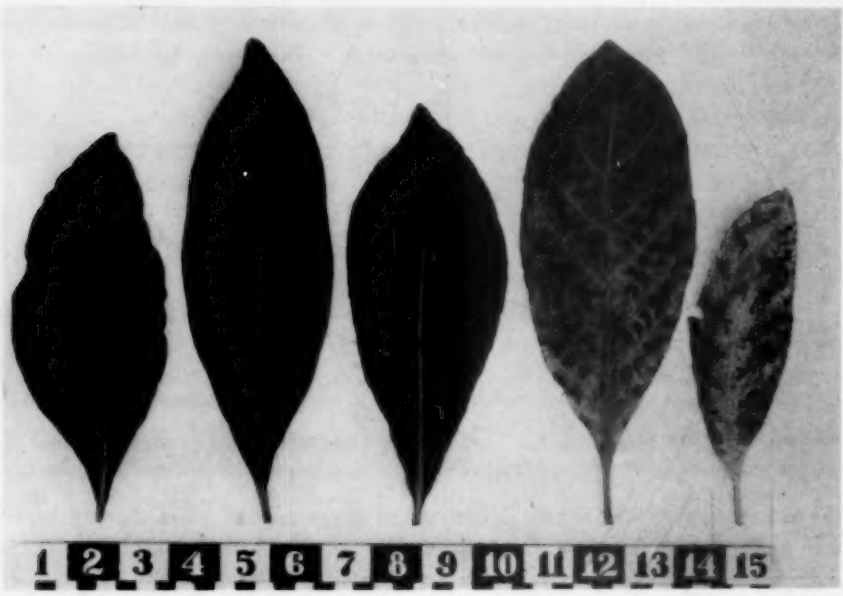

FIG. 3. Leaves of Cinchona showing from left to right progressive stages of disease symptoms on plants subjected to continuous high-soil-moisture conditions.

relatively high, a situation similar to that occurring in most commercial cinchona plantations.

About three months after the treatments were started, small yellow spots which later became necrotic appeared systematically between the veins of leaves of plants in the high-soil-moisture plots (figs. 2 and 3). This disorder which somewhat resembles magnesium deficiency (**2**) or manganese toxicity in other plants later became more pronounced and occurred on nearly all the plants in the high-soil-moisture treatment. Some of the plants lost as much as three-fourths of their foliage. A few of the plants in the medium- and low-soil-moisture plots showed these symptoms but the incidence was less than one-tenth that in the high-soil-moisture plots. This disorder often has been observed on young cinchona trees in the station planting at Toro Negro at 3,200 feet elevation. It is not certain if the disease is pathological or physiological or a combination of both, but it is clear that a high-soil-moisture condition is associated with it.

It is evident from data in table I that seedlings grown at the low-soil-

TABLE I

SURVIVAL AND GROWTH OF *Cinchona ledgeriana* UNDER LOW-, MEDIUM-, AND HIGH-SOIL-MOISTURE CONDITIONS

SOIL MOISTURE TREATMENT	AV. HEIGHT	AV. FRESH WEIGHT	AV. DRY WEIGHT	DRY MATTER	SURVIVAL	TOP-ROOT RATIO
	cm.	*gm.*	*gm.*	%	%	
Low (9 to 13%)	38.9	23.9	6.3	26.4	95.8	6.3
Medium (18 to 24%)	56.4	45.8	10.2	22.2	97.9	8.4
High (30% to field capacity)	64.3	45.7	9.9	21.7	95.8	7.0

moisture level made less growth as measured by height and fresh and dry weights than seedlings grown at medium- and high-soil-moisture conditions. These differences were significant at the 1% level. As might be expected, the percentage of dry matter in the plants grown in low soil moisture was significantly higher than that in plants from the two other treatments. The differences in height and fresh and dry weights between plants grown in medium and high moisture were not statistically significant.

Survival was good and fairly uniform in all treatments; it was slightly but not significantly higher in the medium-soil-moisture plots. The top-root ratios of the plants grown in medium and high soil moisture were not significantly different from each other, though both were significantly greater than the top-root ratio of plants grown under low-soil-moisture conditions.

The total alkaloid and quinine-sulfate content of the roots and stems are given in table II. The roots of plants grown under medium- and high-soil-moisture conditions contained significantly higher total alkaloid and quinine sulfate than the roots of plants grown under low-soil-moisture con-

TABLE II

TOTAL ALKALOID AND QUININE SULFATE IN ROOTS AND STEMS OF *Cinchona ledgeriana* GROWN UNDER LOW-, MEDIUM-, AND HIGH-SOIL-MOISTURE CONDITIONS

SOIL MOISTURE TREATMENT	TOTAL ALKALOIDS		QUININE SULFATE	
	STEMS	ROOTS	STEMS	ROOTS
	%	%	%	%
Low (9 to 13%)	1.90	3.49	0.99	1.77
Medium (18 to 24%)	1.87	4.00	0.83	1.84
High (30% to field capacity)	1.78	3.87	0.96	1.90

ditions. There was no statistically significant differences in the total alkaloid content of the stem nor of the quinine sulfate content of the stems and roots of the plants in the three treatments.

EFFECT OF SOIL MOISTURE ON MINERAL COMPOSITION

From the data presented in table III it is apparent that the soil moisture treatments had a moderate effect on the mineral composition of the leaves. The percentages of ash, calcium, and magnesium were somewhat higher in the leaves from plants grown under low-soil-moisture conditions than in those from plants grown under medium- and high-soil-moisture conditions. Leaves from plants grown under high-soil-moisture conditions contained the lowest percentage of ash, calcium, and magnesium, while leaves of plants from the medium-soil-moisture plots were intermediate with respect to these constituents. The reverse situation was true, however, with respect to the nitrogen, phosphorus, and potassium contents of the leaves. Leaves from plants grown in low-soil-moisture plots contained significantly the least of these elements. There was no significant difference between the contents of these elements in leaves from plants grown in high- and medium-soil moisture.

TABLE III

MINERAL COMPOSITION OF *Cinchona ledgeriana* LEAVES FROM PLANTS GROWN UNDER HIGH-, MEDIUM-, AND LOW-SOIL-MOISTURE CONDITIONS*

SOIL MOISTURE TREATMENT	ASH	TOTAL NITROGEN	PHOSPHORUS	POTASSIUM	CALCIUM	MAGNESIUM
	%	%	%	%	%	%
Low (9 to 13%)	4.99	1.86	0.19	0.71	0.77	0.55
Medium (18 to 24%)	4.65	2.21	0.23	1.02	0.69	0.43
High (30% to field capacity)	4.23	2.35	0.25	1.09	0.57	0.41

* Expressed as percentage of dry weight.

Summary

1. Seedlings of *Cinchona ledgeriana* were grown in greenhouse chambers under high-, medium-, and low-soil-moisture conditions at Mayaguez, Puerto Rico. The temperature was automatically controlled and the humidity was maintained relatively high.

2. Leaves of seedlings grown under high-soil-moisture conditions developed necrotic spots between the veins and many of them eventually yellowed and abscissed. This disorder, which resembles magnesium deficiency or manganese toxicity in other plants, has been commonly observed in the station field plantings at Toro Negro National Forest, Puerto Rico.

3. Seedlings grown under low-soil-moisture conditions were significantly shorter with a lower top-root ratio and less fresh and dry weight, but a higher percentage dry matter than seedlings grown under either medium- or high-soil-moisture conditions.

4. Total alkaloid and quinine sulfate in roots of seedlings grown in medium and high soil moisture were significantly higher than those in roots of seedlings grown in low soil moisture.

5. Percentages of ash, calcium, and magnesium were highest in plants grown in low soil moisture and lowest in plants grown in high soil moisture. The nitrogen, phosphorus, and potassium contents, however, were significantly the least in plants grown in low soil moisture.

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DISTRIBUTION OF THIAMIN AND RIBOFLAVIN IN THE TOMATO PLANT[1]

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The vitamin content of plant material has been extensively studied during the last few years but these studies have dealt almost exclusively with single organs of a plant, such as fruits or other edible part, and only a few have been concerned with the distribution of these vitamins within the plant. The investigations that have had as their objective the determination of the quantity of a particular vitamin in different parts of a plant have, in general, shown that the young parts contain more vitamin than the older parts. LAMPITT, BAKER, and PARKINSON (**8**) found that ascorbic acid was present in highest concentration in young leaves and young tubers of the potato plant. RYTZ (**9**) found that in the pea plant there was a decrease in thiamin as the plant aged, the leaves always containing more than the stem, which had a higher concentration than the roots. Flower buds were on par with young leaves. BURKHOLDER and MCVEIGH (**3**) observed that in corn seedlings the embryo leaves contained most thiamin and, as the leaves became older, there was a decrease. The same authors (**4**) found that thiamin, riboflavin, niacin, and B_c (the antianemic factor) increased in the buds of trees as they opened but again there was a decrease as the leaves matured. On the other hand, BURKHOLDER and SNOW (**5**) noted that in forest trees the buds contained more thiamin than the leaves. BONNER (**1**) and BONNER and DORLAND (**2**) found that the youngest tomato leaves contained the highest concentration of thiamin, riboflavin, and pantothenic acid. There was also a gradient in the stem with the younger part having more vitamin than the older part. The roots had a higher concentration of riboflavin and pantothenic acid than any part of the stem, but thiamin was present in a higher concentration in the young stem than in the root.

The present work has as its purpose to extend the work on distribution of vitamins in the tomato plant.

Methods

The method developed by CONNOR and STRAUB (**6, 7**) for combined riboflavin and thiamin determination has been used throughout the work, with only some slight modifications necessary in the use of plant material. In this method the plant material is first homogenized and then extracted with 0.05 N H_2SO_4 solution at a temperature of boiling water for a period of one hour with occasional stirring. The extract is digested with clarase at

[1] Paper from the Department of Botany of the University of Michigan, no. 846. This investigation has been aided financially by the Horace H. Rackham Trust Fund of the University of Michigan.

a pH of 4.5 for two hours at a temperature of 45° C. to break down the cocarboxylase which is a pyrophosphate ester of thiamin. Next the digest is centrifuged for 45 minutes at 2,500 rpm. Twenty-five milliliters of the clear solution is slowly dropped, first onto activated Decalso then onto Florisil; the former adsorbs the thiamin and the latter the riboflavin. The adsorbents are held in place by a small amount of glass wool in 15-milliliter special tubes, and the tubes are so arranged that the solution runs from the one with Decalso into the one with Florisil. After the solution has run through the tubes the adsorbents are washed with 35 ml. of hot distilled water. The tubes are next separated and the thiamin is eluted with hot 25% KCl to a volume of 40 ml. Five-milliliter aliquots are oxidized with alkaline potassium ferricyanide to thiochrome, which is taken up in 15 ml. of isobutyl alcohol. The isobutyl alcohol is separated by centrifugation, pipetted off, and any water removed with anhydrous sodium sulfate. The fluorescence of the thiochrome contained in the isobutyl alcohol is determined by a lumetron fluorimeter. The isobutyl alcohol, clarase, and perhaps the plant material itself, contains some material fluorescing in ultraviolet light; thus an unoxidized, as well as an oxidized, aliquot is run from each sample. The fluorescence of the unoxidized aliquot is subtracted from that of the oxidized aliquot and the difference is the fluorescence due to the thiamin present in the plant material. Repeated tests of the clarase have shown it to be free from thiamin. For standardization of the light intensity in the lumetron a quinine sulfate solution having a concentration of 27 mcg./ml. is used.

The adsorbed riboflavin is eluted with 20% pyridine-2% acetic acid solution and made up to a volume of 50 ml. Aliquots of 15 ml. are pipetted into brown Erlenmeyer flasks and the foreign substances which may also fluoresce are oxidized by 1% potassium permanganate, the excess of which is destroyed by 3% hydrogen peroxide. The riboflavin is determined in the lumetron by its yellow-green fluorescence in blue light. It has been found that the clarase contains some riboflavin and therefore a control is run with clarase and its fluorescence in blue light is subtracted from the plant sample to get the fluorescence due to the riboflavin in it. For standardization of the light intensity a 0.1 mcg./ml. solution of sodium fluorescein is employed. All of these determinations must be done in subdued light because riboflavin is destroyed by light.

Calculations are made by the use of standard curves obtained by the use of known concentrations of thiamin and riboflavin.

The plant material has been the John Baer tomato grown in the greenhouse. As the experiments have extended over a considerable period of time the plants have been exposed to different light conditions, but as no comparisons are made between different experiments this does not matter.

Results

In this investigation attempts have been made to gain information on the general distribution of thiamin and riboflavin in the tomato plant. In the

first few experiments the apical part of the plant, including the immature stem and leaves, and mature leaves were used. Later a more extensive examination of the plant was made in which leaves of four different ages, obtained from different levels of a tall plant, were employed. Both of these sets of experiments are recorded in Section One of table I. It will be noted that the immature leaves and stem apex contain the most thiamin and riboflavin, and there is a gradual decrease in the concentration of these vitamins

TABLE I

THIS TABLE IS A SUMMARY OF A NUMBER OF EXPERIMENTS IN WHICH VARIOUS COMPARISONS AS TO CONCENTRATIONS OF THIAMIN AND RIBOFLAVIN HAVE BEEN MADE. THE FIGURES DENOTE MICROGRAMS PER GRAM OF FRESH MATERIAL. DIFFERENT GROUPS OF EXPERIMENTS ARE REFERRED TO AS SECTIONS FOR READY REFERENCE TO THE TEXT

SECTION	DATE	PART OF PLANT USED	THIAMIN	RIBOFLAVIN
I	12–13	Immature leaves and stem	1.5	2.67
			1.9	3.22
	12–20	Mature leaf, (only blade)	1.2	3.50
		Whole mature leaf	0.46	2.17
		Whole leaves from different parts of stem		
	5–4	a. Immature leaves	3.69	1.57
			2.26	2.83
	5–6	b. Mature leaves—18 in. below *a*	1.07	1.47
			0.92	1.82
		c. Mature leaves—15 in. below *b*	0.23	1.34
			0.67	1.20
		d. Pale green with yellow markings	0.77	1.12
			0.08	1.01
II	1–15	Only leaf blade—mature leaf	0.29	2.05
			1.61	1.20
	2–11	Rachis and petiole	0.00	0.50
			0.04	0.32
		Stem near leaf insertion	0.06	0.50
			0.31	0.45
III	2–5	Apical part of plant	1.22	1.60
			1.23	2.05
	6–8	Middle part of plant	0.46	0.86
			0.27	1.01
		Roots	0.15	1.02
			0.23	0.99
IV	4–13	Leaves	0.77	1.41
			0.92	0.77
	4–15	Ripe fruits	0.73	0.53
			0.46	0.48

as the leaves age. An apparent exception to this is the result for the experiment of May 4, in which the concentration of the thiamin is higher in the oldest leaves than in the ones just preceding them. The reason for this is that the oldest leaves used in this experiment were very old and some were partly dead with dry margins. This would increase the dry weight. Even under these conditions the riboflavin showed a steady decrease with the age of the leaves. These experiments are confirmations of BONNER's findings (**1**) some years ago for the tomato plant, and agree with investigations of other plants where it has been shown that the young parts of a plant are richest in vitamins.

In the experiment of December 13, Section One of table I, only the leaf blade was used of the mature leaf and the thiamin content was essentially the same as that of the immature leaf and stem and the riboflavin concentration was even higher, whereas in another experiment run a few days later the whole mature leaf was quite low in these vitamins, thiamin especially. This led to an investigation of the distribution within the leaf and the stem to which the leaf was directly attached. Section Two of table I shows two of these experiments; the second one was run 13 months later than the first one. It is to be observed that the rachis and petiole contain no or very little thiamin and less riboflavin than the blade. The thiamin concentration was very low in the first experiment and that may account for the lack of the vitamin in the rachis and petiole in this experiment; in the later experiment, however, the thiamin was quite high in the blade and yet the rachis and petiole were practically devoid of thiamin. The difference in the riboflavin concentration was somewhat less in the different parts. These experiments demonstrate clearly that the blade is richer in thiamin and riboflavin than either the rachis and petiole of the leaf or the stem to which the leaf is directly attached.

It was also desirable to investigate the roots. For this purpose plants grown in nutrient solution were used. The two experiments cited under Section Three of table I were made on plants 13–16 inches tall in the first experiment and 24–26 inches tall in the second experiment. The second lot of plants were about three months old. Taking the two experiments together the roots contain a little less thiamin than the leaves and stems of the middle portion of the plant and much less than the apical part, but the riboflavin is not essentially different in the roots and the middle portion, though here also the apical part is much richer in vitamin. The two experiments cited in table I were made four months apart and the results are essentially the same, though the plants were grown under different light and temperature conditions and the plants in the later experiment were more vigorous than those in the earlier one.

One more group of experiments was set up to examine the relation between vitamins and tissue. In this group of experiments immature leaves and stems were obtained from the main apex of plants of different age-groups. The results are recorded in table II. The youngest plants always contained the lowest concentration of both thiamin and riboflavin, but the former showed a much greater difference. It is very surprising that the youngest plants, which have the highest physiological activity, had the lowest vitamin content. This can be explained in a number of ways. One might consider that a precursor for the vitamin is built up slowly and in some other part of the plant and as the plant becomes larger it produces more of this substance which is then activated in the young part of the plant. It could also be considered that a larger and older plant has accumulated more stored material which is used in the synthesis of the vitamins. As the days get longer and lighter in the spring it takes less time for this ma-

TABLE II

THIS TABLE SHOWS A COMPARISON OF THE CONCENTRATION OF THIAMIN AND RIBOFLAVIN IN THE TIPS (IMMATURE STEMS AND LEAVES) DERIVED FROM PLANTS OF DIFFERENT AGES. AGE OF THE PLANTS IS GIVEN IN MONTHS AT TOP OF COLUMNS, AND THE AMOUNT OF VITAMIN IN MICROGRAMS PER GRAM FRESH MATERIAL. THE FIGURES WITHIN THE BRACKETS BELOW VITAMIN VALUES ARE THE HEIGHTS OF THE PLANTS IN INCHES

DATE	THIAMIN					RIBOFLAVIN				
	1	2	3	4	5	1	2	3	4	5
1–10	.....	0.03 (5)	0.75 (26–30)	1.12 (60–64)	.....	.....	1.36	1.74	1.87	.....
2–20	0.54 (5–6)	.....	1.33 (22–23)	2.20 (50–60)	.....	1.33	.....	1.87	1.89	.....
4–20	.....	1.11 (15–19)	1.84 (36–42)	.....	2.61 (72–80)	.....	1.55	1.63	.....	1.34
4–22	1.30 (4–5)	.....	2.45 (36–42)	2.26 (52–64)	2.53 (72–80)	1.54	.....	2.24	2.40	1.68

terial to accumulate and in April it has reached a sufficient level in three-months-old plants, whereas that was not true in January when the days were short and dark.

Most of the data available on the tomato in the literature is on the fruit and as these experiments are concerned with the distribution in the whole plant a comparison between fruits and leaves has also been made. Section Four in table I shows two such experiments made in April. In one experiment the difference is very large but in the other it is small as to the thiamin concentration, but the riboflavin is always much more abundant in the leaves than in the fruits. Experiments made in January give a greater difference in both vitamins between the leaves and fruit than the later experiments. These findings are a little different from those reported by WILSON and WITHNER (**10**) who found that the thiamin concentration was always greater in the fruits than in the leaves and roots, but conversely the leaves had more riboflavin than the fruits. They used five varieties of tomato but not John Baer.

Discussion

While no experiments have been made to determine the effect of light on the formation of the thiamin and riboflavin there seems to be considerable evidence that light influences the synthesis of thiamin. In nearly all instances where determinations were made several months apart those made later when the days were longer and brighter had the higher concentration. This is specially well brought out in table II. No light measurements were made so naturally one cannot speak too authoritatively in this connection, but the general trend makes one suspect that light furthers the synthesis of thiamin, either directly or indirectly. The light does not seem to influence riboflavin nearly so much.

The great difference in thiamin and riboflavin concentration in the apical region of plants of different ages is very puzzling but as explained earlier it must be due to a larger amount of precursor produced in the larger plants. If this precursor or perhaps merely labile food material is conducted to the growing regions where the thiamin is synthesized the larger the photosynthesizing area the more of this material will be available. Undoubtedly there would be reached an age or size after which there would be no further increase as the capacity of the synthesizing region has also been reached. It will be seen from table II that as far as the riboflavin is concerned this seems to have been reached in plants three months old in the April experiments, though perhaps not in the thiamin synthesis. This view is further strengthened by the fact that as more light becomes available for photosynthesis the difference between the young and old plants becomes less.

There seems to be very little doubt as to the tissue in which the synthesis takes place. The leaves are much richer in vitamins than any other part of the plant and when the leaf is divided into blade, rachis, and petiole, the blade has practically all of the vitamin of the whole leaf. One might then think that there is a close relationship between photosynthesis and

thiamin synthesis, but that need not be true. Leaves are active growth centers and there is no reason why vitamin synthesis should not also take place there, even though it is not in any way connected with photosynthesis.

Summary

The distribution of thiamin and riboflavin in the tomato plant has been investigated, and it has been found that:

1. The immature leaves and stem have the highest concentration of these vitamins.
2. In mature leaves practically all of the vitamin is in the blade of the leaf.
3. The roots have as much riboflavin as the middle part of the plant but much less than the apical part of the plant.
4. The roots have considerably less thiamin than the middle part of the plant.
5. There is an increase in the thiamin and riboflavin in the tip of the tomato plant as it increases in age or size, at least up to 4 to 5 months.
6. The mature leaves contain more of these vitamins than do the ripe fruits.

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THE ABSORPTION OF PHOSPHORUS AND IRON FROM NUTRIENT SOLUTIONS[1]

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(WITH FIVE FIGURES)

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Two of the more popular solutions for experimental culture of plants show a striking variation in the amounts of phosphorus used. Shive's so-called best solution, R_5–C_2 (**4**) contains 2.45 gm. per liter or 0.018 mols of KH_2PO_4, partly as a source of phosphorus, but more perhaps as an acid buffer tending to maintain the solution at pH 4.5–5.0. Hoagland's 1940 solution (**2**) on the other hand contains only 0.068 gm. per liter or 0.0005 mols of the same salt. The Shive solution thus contains 36 times as much phosphorus as the equally successful Hoagland solution. As a portion of a general study of ion balance in nutrient solutions we have compared the growth, color and phosphorus absorption of several plants in these mixtures and in two experimental solutions; the first ("X") somewhat resembling the Hoagland solution, but carrying more than half of its nitrogen as NH_4NO_3, and the second ("P") being a modification of Zinzadzé's buffered solution (**6**) carrying a moderately high concentration of phosphorus as a colloidal precipitate of the tricalcium salt and with the nitrogen again added as NH_4NO_3.

Methods

Plants were grown in quart mason jars with cork stoppers (**3**). Four seedlings in each jar were thinned to two to obtain maximum uniformity. Five replicates were used for each treatment and some of the experiments were repeated as many as five times. Corn (*Zea mays*), broccoli (*Brassica oleracea* var. *italica*), soybeans (*Glycine max*), tomatoes (*Lycopersicon esculentum*), sunflowers (*Helianthus annuus*), cotton (*Gossypium hirsutum*), and rice (*Oryza sativa*) were used in a main test of growth rates in April and May of 1945; corn, soybeans, and rice were used in June and July of the same year for studies of phosphorus absorption. Some of the plants were grown to fruiting, but, because of the small size of the culture jars, most of the work, including the phosphorus analyses, was done with plants 4 to 6 weeks old.

Concentrations of the salts used in grams per liter and of the various ions in millimols are shown in table I. Iron was furnished by 10 ml. of a 0.1% solution of ferric tartrate at each change of solutions and by 1.0 ml. additions of the same solution one to three times a week as required. All solutions received 1.0 p.p.m. of boric acid and 0.5 p.p.m. of $ZnSO_4$ at each change. After the seedling period of 2–3 weeks, solutions were changed at

[1] Journal Paper No. J–1434 of the Iowa Agricultural Experiment Station. Project 896.

TABLE I

CONCENTRATIONS OF SALTS IN GRAMS PER LITER AND OF IONS IN MILLIMOLS FOR FOUR NUTRIENT SOLUTIONS

SALT OR ION	HOAGLAND 1940 ("H")	SHIVE R_5–C_2 ("S")	EXP. SOL. "X"	EXP. SOL. "P"
	gm./l.	*gm./l.*	*gm./l.*	*gm./l.*
NH_4NO_3	.....	.....	0.25	0.286
KNO_3	0.506	.....	0.25	.....
$Ca(NO_3)_2$	0.591	1.228	0.25	.....
KCl	.....	.....	0.25	0.50
$MgSO_4$	0.247	3.697	0.50	0.50
$Ca_3(PO_4)_2$	.....	.....	.....	1.00
KH_2PO_4	0.068	2.450	0.10	.....
Total	1.412	7.375	1.60	2.286
	m. mols	*m. mols*	*m. mols*	*m. mols*
Ca^{++}	2.5	5.2	1.1	3.2
Mg^{++}	1.0	15.0	2.0	2.0
K^+	5.5	18.0	6.6	6.7
NH_4^+	.....	.....	3.1	3.6
NO_3^-	7.5	10.4	9.7	3.6
SO_4^{--}	1.0	15.0	2.0	2.0
HPO_4^-	0.5	18.0	0.7	3.2

weekly intervals, except that solutions for larger plants (6–10 weeks) were changed at intervals of 3–5 days.

GROWTH OF PLANTS

The size ranking of plants started April 4–11 was estimated on May 10 and again on May 23. Differences between the best and poorest cultures

TABLE II

RANKING FROM BEST TO POOREST OF SEVEN SPECIES IN FOUR SOLUTIONS. EXPERIMENT STARTED WEEK OF APRIL 4

PLANT	RANK ON BASIS OF TOP AND ROOT GROWTH			
	FIRST	SECOND	THIRD	FOURTH
May 10, 1945				
Corn	H	X	P	S
Broccoli	P	X	H	S
Soybeans	P	X	S	H
Tomatoes	P	X	S	H
Sunflowers	P	X	H	S
Cotton	P	S	H	X
Rice	P	X	S	H
Scores	P 26	X 19	H 12	S 12
May 23, 1945				
Corn	H	P	S	X
Broccoli	X	P	H	S
Soybeans	P	X	H	S
Tomatoes	P	X	S	H
Sunflowers	P	H	X	S
Cotton	P	H	S	X
Rice	X	P	S	H
Scores	P 25	X 18	H 16	S 11

were slight in tomato to marked in soybean and broccoli (fig. 1). Rankings together with a score obtained by assigning a value of 4 for first rank, 3 for second, etc., are shown in table II, and six of the plants are shown in figure 1.

On the basis of the scores in table II the "P" solution containing NH_4NO_3 and $Ca_3(PO_4)_2$ was the best for the plants tested, while Shive's R_5–C_2 ("S")

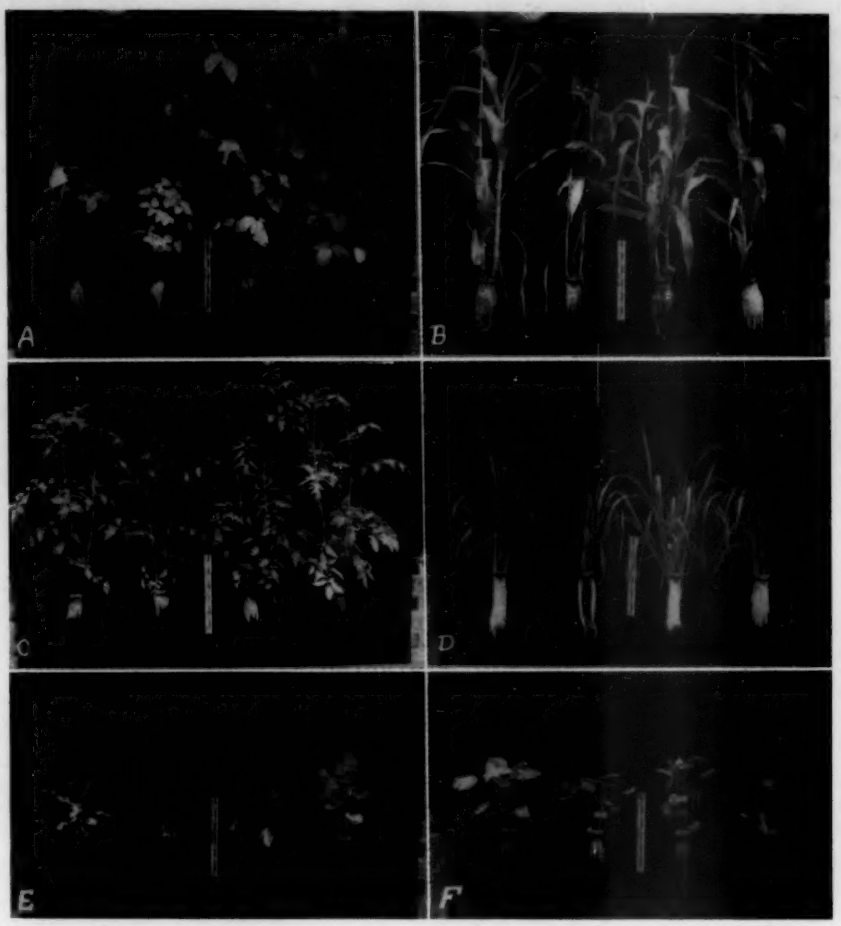

FIG. 1. Six species grown in four nutrient solutions. A, soybeans; B, corn; C, tomatoes; D, rice; E, broccoli; F, cotton. Left to right in each photograph: Hoagland's solution ("H"), Shives R_5–C_2 ("S"), a buffered solution ("P") and an unbuffered solution with NH_4NO_3 ("X"). Plants started April 4–11, photographed June 15.

rated only one second place in the upper brackets. There was much evidence, however, that the "best" solution changes with the light and temperature conditions (**1**), with the plant, and perhaps most rapidly with the age of the plant. "X" was a good solution for young corn plants, but they soon developed root rots in the excessive acidity produced. On the other hand, larger plants did reasonably well when returned to this solution (fig. 1B).

Small corn in Hoagland's ("H") solution is chlorotic and may die unless especial attention is given to the iron supply. Even badly chlorotic plants recover quickly, however, if the phosphorus is omitted from the solution for the first half of the week. Figure 2A shows this effect. The solutions were identical except for the time of adding phosphorus. Soybeans also do poorly in "H" solution, but can be carried by delaying the phosphorus (fig. 2B). This principle was used by WEISS (**5**), working in this laboratory. Chlorosis was induced in genetic lines of soybeans to be tested for efficiency of iron absorption by adding moderate quantities of KH_2PO_4 to a large culture tank. A differential chlorosis developed among the lines which disappeared as the phosphorus was exhausted and more iron was added to the solution.

FIG. 2. Effect of alternating phosphorus and iron in Hoagland's solution. A, corn; B, soybeans; left in each photograph, phosphorus and iron added together at solution changes; right, phosphorus addition delayed 2–4 days to permit unhindered iron absorption.

The effect of phosphorus in causing chlorosis in "H" but not in "S" solution, which contains 36 times as much phosphorus, is explainable on the basis of pH. The pH's of fresh and used solutions in table III show that the large quantity of KH_2PO_4 tended to maintain an acid reaction favorable to iron absorption. Modified Shive solutions containing intermediate concentrations of acid phosphate are less successful. In Shive's original paper (**4**), solutions in the R_1 series contained KH_2PO_4 at 0.0036 mols; series R_2 at twice this concentration, R_3 at three times, etc., to R_7. None of the R_1 or R_2 solutions was rated good while R_4's and higher were mostly good to excellent, and three solutions, R_3–C_3, R_4–C_5, and R_7–C_2, were not significantly poorer than R_5–C_2.

TABLE III

HYDROGEN ION CONCENTRATIONS (pH) OF FRESH AND USED NUTRIENT SOLUTIONS

SOLUTION	FRESH SOLUTION	USED SOLUTION, 3–4-WEEK PLANTS	USED SOLUTION, 5–6-WEEK PLANTS
Hoagland 1940	5.20	5.80–6.35	5.40–6.55
Shive R_5–C_2	4.45	4.85–5.65	4.45–5.70
Exp. sol. "X"	5.25	3.55–4.40	3.70–5.70
Exp. sol. "P"	6.10	5.50–6.30	5.15–5.60

Ammonium nitrate was added to solutions "X" and "P" as a neutral source of nitrogen. We were very considerably surprised, therefore, to find that the pH of the unbuffered solution "X" dropped as low as pH 2.9 and always went below 4.0. The cause of the acidity was shown to be the preferential absorption of ammonia ions by all of the plants studied at all stages of growth up to early fruiting. Ammonia was absorbed faster than NO_3^- from the unbuffered "X" solution, the buffered "P" solution or pure NH_4NO_3. Corn and cotton roots (fig. 1F) were injured in these acid solutions but soybeans made excellent growth with a very dark green color (fig. 1A). Young plants sometimes became chlorotic in "X" because they did not absorb nitrogen fast enough to develop an acid reaction. With large plants nitrogen absorption was so rapid that the acid phase was ended on the second or third

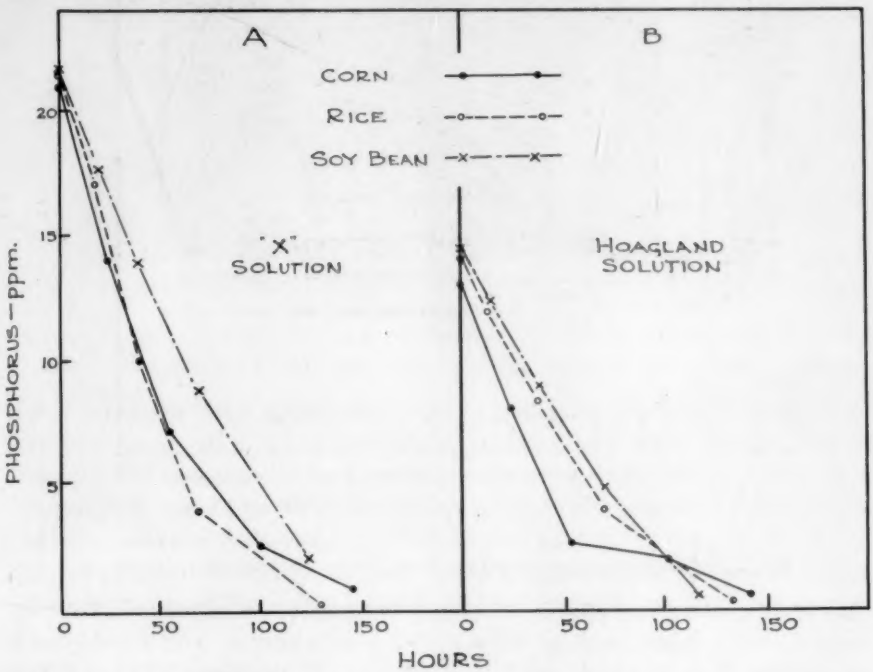

FIG. 3. Phosphorus absorption by three species grown in "X" and Hoagland solutions.

day by nearly complete absorption of both NH_4^+ and NO_3^-. In the "P" solution the $Ca_3(PO_4)_2$ prevented low pH values. At the lower pH's shown for this solution in table III, phosphorus became more soluble (fig. 5) and some tendency toward chlorosis was observed.

The absorption of phosphorus

The apparent interaction of phosphorus and pH in iron chlorosis led to a detailed study of PO_4 absorption from the four solutions. The results are shown graphically in figs. 3, 4, 5. The "H" and "X" solutions (figs. 3A and B) starting respectively with 14 and 21 p.p.m. of phosphorus dropped to 1 p.p.m. or less in 5 or 6 days. Essentially all of the 0.068 or 0.100 gm.

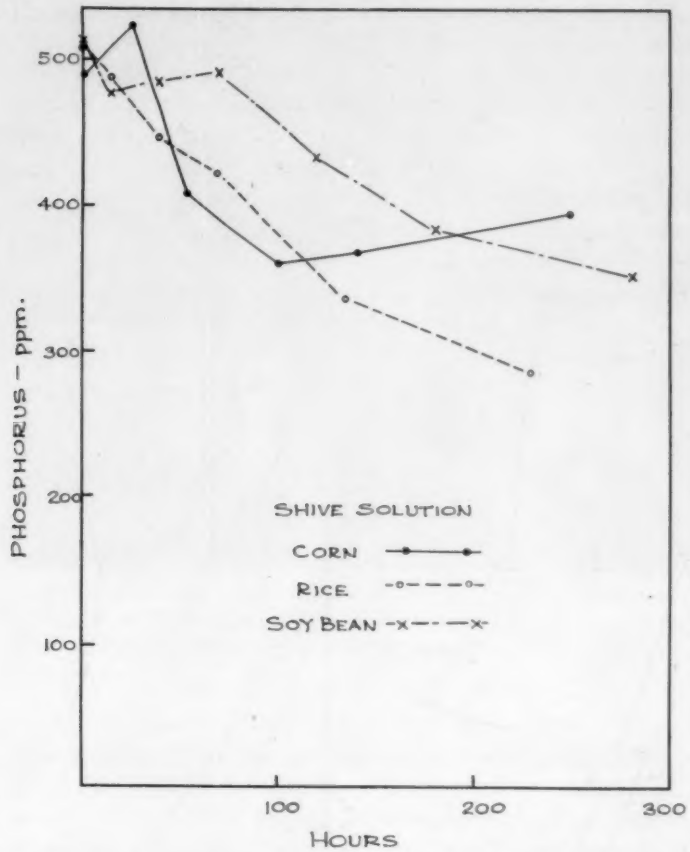

Fig. 4. Phosphorus absorption by three species grown in Shive solution.

of KH_2PO_4 added was absorbed. The 500 p.p.m. of phosphorus in the "S" solution was reduced to about 300 p.p.m. in a week (fig. 4) with a phosphorus absorption 10 or 15 times that in the "X" and "H" solutions. The fact that such heavy absorption did not seriously interfere with iron utilization is evidence that the interference observed with the "H" solution occurred out-

side the plant. Soluble phosphorus concentrations in the "P" solution, which contained a large excess of colloidal $Ca_3(PO_4)_2$, started at about 10 p.p.m. and climbed to 20–30 p.p.m. on the second and third days (fig. 5) as some of the insoluble phosphate was brought into solution by accumulating HNO_3 from the differential utilization of NH_4NO_3. Soluble phosphorus then tended downward but remained above 10–20 p.p.m. after 10 days. The "P" solution has been best for small plants with slow NH_4^+ absorption so that the pH is held near 6.0. Very little iron chlorosis develops in these cultures in

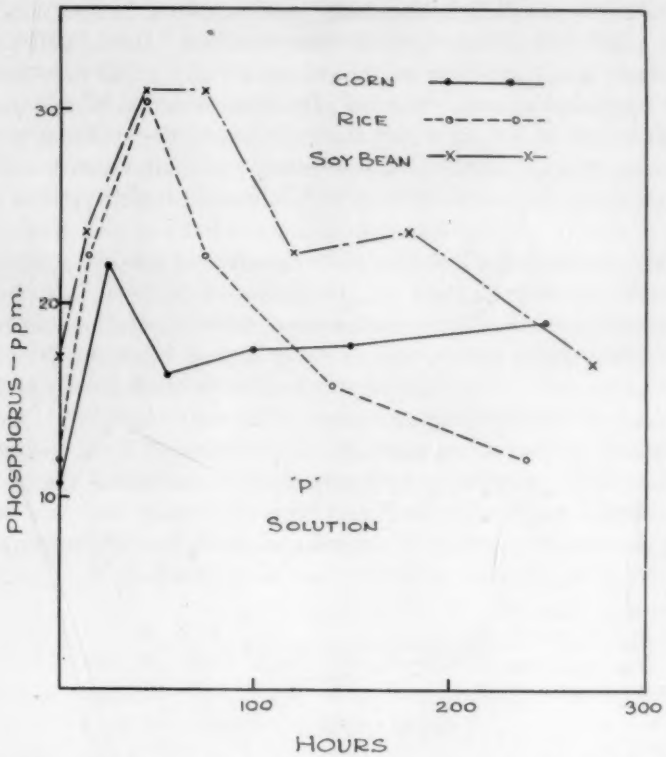

FIG. 5. Phosphorus absorption by three species grown in the $Ca_3(PO_4)_2$ buffered "P" solution.

spite of the high pH and phosphorus, suggesting that it is the dibasic or more probably the monobasic phosphate ion which precipitates the iron. The "P" solution would probably be improved for older plants by using some $Ca(NO_3)_2$ along with $Ca(Cl)_2$ in forming the $Ca_3(PO_4)_2$. The result would be a partial replacement of KCl by KNO_3, a higher total nitrogen and perhaps less tendency for excess solubility of the phosphorus.

Discussion and summary

Moderate amounts of KH_2PO_4 (0.5 to 0.7 millimols) caused iron chlorosis of seedlings in solutions less acid than pH 5.5–6.0. The trouble could be

avoided by omitting the phosphorus entirely and adding it separately after 2–4 days. Even with this modification the Knop type of solution (Hoagland, **2**) caused persistent chlorosis in soybeans and broccoli.

In the commonly used Shive solution (R_5–C_2) a large excess of KH_2PO_4 holds the pH of the solution between 4.5 and 5.0 and chlorosis is moderate to slight. The Shive solution contains 36 times as much phosphorus as the Hoagland and 6-week-old plants growing in it absorbed 15 times as much to make a slightly poorer growth. The addition of as little as 0.125 gm. per liter of NH_4NO_3 resulted in decidedly acid solutions in every experiment with nine plant species representing seven families. Initial pH's of 5.25 in an unbuffered solution fell to minimums of 3.9 to 2.9 and then rose, to pH 5.0 or 6.0 with large plants. The low pH's were shown to be due to a preferential absorption of NH_4^+ ion and the later rise to the slower absorption of the NO_3^- ion. The use of NH_4NO_3 prevented iron chlorosis with some plants, notably soybeans, but resulted in acidities which were injurious to young corn and to cotton. In work done in this series but not described above, the "X" solution containing NH_4NO_3 gave exceptional growth of young coffee (*Coffea arabica*), but was toxic to older plants.

It is probable that iron chlorosis has more effect on solution culture results than any other single factor, and frequently than all other factors. Iron absorption from cultures is reduced by phosphorus, probably by $H_2PO_4^-$ ions especially, at pH's of about 6.0 or higher. The use of $Ca_3(PO_4)_2$ reduces the trouble as does the use of enough KH_2PO_4 to maintain a pH below 5.5. In tank culture H_2SO_4 may be used more cheaply to accomplish the same result. In miscellaneous work with the Knop type of solution chlorosis can be reduced or prevented by omitting phosphorus from the solution and adding it separately 2–4 days later after iron has been absorbed.

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BRIEF PAPERS

SELECTIVITY OF 2,4-D AND SINOX WHEN APPLIED TO SOIL

L. L. DANIELSON

(WITH ONE FIGURE)

Received July 18, 1947

The use of 2,4-D (2,4-dichlorophenoxyacetic acid) and Sinox (sodium dinitro ortho cresylate) for the control of weeds in broadleaf crops has not proved feasible in any general way. Evidence is accumulating, however, to show that certain crops may be treated with these materials in some stages of development, and in some cases during dormancy, without producing apparent injury. Crop plants of this type, however, are very limited in number.

The application of these chemicals to the soil to control weeds in field seed beds seemed to present a possibility for extending their use. Accordingly, a preliminary experiment was conducted in 1946 to canvass the possibilities in such a method of application.

The trials were conducted on a Sassafras loam prepared as for seeding corn or lima beans. Sinox in concentrations of one to four gallons per acre and 2,4-D (70% sodium salt) in concentrations of 1 to 4 pounds per acre were applied as a spray on this freshly prepared soil. The chemicals named were applied on plots 8 by 50 feet square in sufficient water to provide 500 gallons per acre to make possible the uniform distribution of the chemicals.

TABLE I

EFFECT OF SOIL TREATMENTS ON WEED POPULATIONS EXPRESSED AS NUMBER OF BROADLEAF AND GRASS PLANTS PER SQUARE YARD*

SINOX—GALS. PER ACRE	1	2	3	4
Broadleaf plants				
Av. 3 areas	47.3	8.3	2.6	0.0
% control	46.1	90.6	96.8	100.0
Grass plants				
Av. 3 areas	169.6	148.3	98.6	75.6
% control	10.4	21.7	11.6	31.5
2,4-D—LBS. PER ACRE	**1**	**2**	**3**	**4**
Broadleaf plants				
Av. 3 areas	26.6	27.6	28.3	40.3
% control	67.0	65.8	81.1	73.1
Grass plants				
Av. 3 areas	16.6	21.0	16.3	9.6
% control	85.0	81.0	88.3	93.1

* Principal broadleaf weed was carpetweed (*Mollugo verticillata*). Principal grass was crabgrass (*Digitaria sanguinalis*). 2,4-D used was the sodium salt in 70% concentration. Soil treated 7/1/46. Populations counts made 8/22/47.

The applications were made in the first week of July 1946 and were evaluated 7 weeks later. The treated areas were not cultivated during this period.

Periodic examination of these plots revealed a striking difference between the plant selectivity of the two chemicals as applied to the soil in the manner described. As is generally known, the selectivities of these materials are very similar when applied to the foliage of growing plants. Evaluation of these plots 7 weeks after treatment, table I, showed that the 2,4-D was toxic to both the grasses and the broadleaf weeds, whereas the Sinox was only slightly toxic to the grasses but very toxic to the broadleaf weeds. Figure 1 shows the effective control of grasses and broadleaves of a 4-pound-per-acre application of the 2,4-D.

FIG. 1. Check plot (1) at left above. Plot (2) at right which received 4 pounds per acre of the sodium salt of 2,4-D (70%) applied to the soil in 500 gallons of water per acre.

The toxic effects of these materials were rather quickly dissipated in the soil as indicated by the normal growth in all treatments of a planting of Henderson bush lima beans and Golden Cross sweet corn made ten days after the chemicals were applied to the soil. The effective rainfall during the interval between treatment and planting was 3.75 inches.

The results of these preliminary trials are considered as indicative rather than conclusive because of their limited nature in terms of weather and soil variations.

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SOLUBLE SOLIDS CONTENT OF DIFFERENT REGIONS OF WATERMELONS

JOHN H. MACGILLIVRAY

(WITH ONE FIGURE)

Received January 27, 1947

Several workers have used a hand refractometer to determine the soluble solids content of watermelons, *Citrullus vulgaris* Schrad. These values appear to be closely related to the sugar content of the melon. Judging from the data of PORTER, BISSON, and ALLINGER (**2**) in Klondike watermelons, 85% of the soluble solids consists of total sugars. Thus, the hand refractometer reading has been used as a measure of quality. An accurate method of determining soluble solids is especially valuable in studying the effect of this quality factor, both in improvement programs and in work on the effect of environment on quality. An accurate method of sampling the melon is also of critical interest. In 1934 TUCKER (**4**) noted the variability of different sections of a watermelon in soluble solids content. Since Tucker's data were limited to observations on one melon, it has seemed desirable to repeat the work, using a larger number of fruits.

In 1938, watermelons from an irrigation experiment were used in this project. Both nonirrigated and heavily irrigated melons of the Klondike variety were included. Previous studies (**1**) have shown that, at Davis, California, irrigation does not greatly affect soluble solids content. All comparisons in this paper were between readings made on the same melon. For the determinations, the melon was placed with the ground spot on a table and cut into equal halves vertically from stem to blossom end. The areas shown in figure 1–B were then sampled. Sample areas in the red flesh were given numbers, whereas those close to seed regions were indicated by small letters of the alphabet. Next, the sampled half was laid on the table with the cut surface up. The melon was cut vertically from stem to blossom end into two quarters. The cut surface of the quarter having the ground spot was then sampled (fig. 1–A). All samples were obtained from the edible flesh even though close to seed or rind. Juice was expressed by hand from a cube of the flesh about one-half inch on each side. Soluble solids content of each sample was determined with a hand refractometer. A scale on this instrument gives the solids percentage of pure sucrose solutions. Readings thus obtained are called, in this paper, "soluble solids." Values found are the averages obtained from twenty-three melons used in this experiment (fig. 1 and table I).

Edible flesh was removed from the two quarters used for the small samples, and the juice was extracted by hand, pressing the flesh in a piece of cheesecloth. The soluble solids content of this juice was considered representative of the whole melon.

In each of the watermelons twenty-three areas were sampled. Table I shows these values, arranged in a descending order from the largest to the smallest. The data indicate a marked decrease in the sugar content as the samples approach the rind from the seed area. High soluble solids are found in the center flesh of the melon, including the surrounding tissue near the seed area. These values which vary from 12.85% to 12.25%, average 12.59%

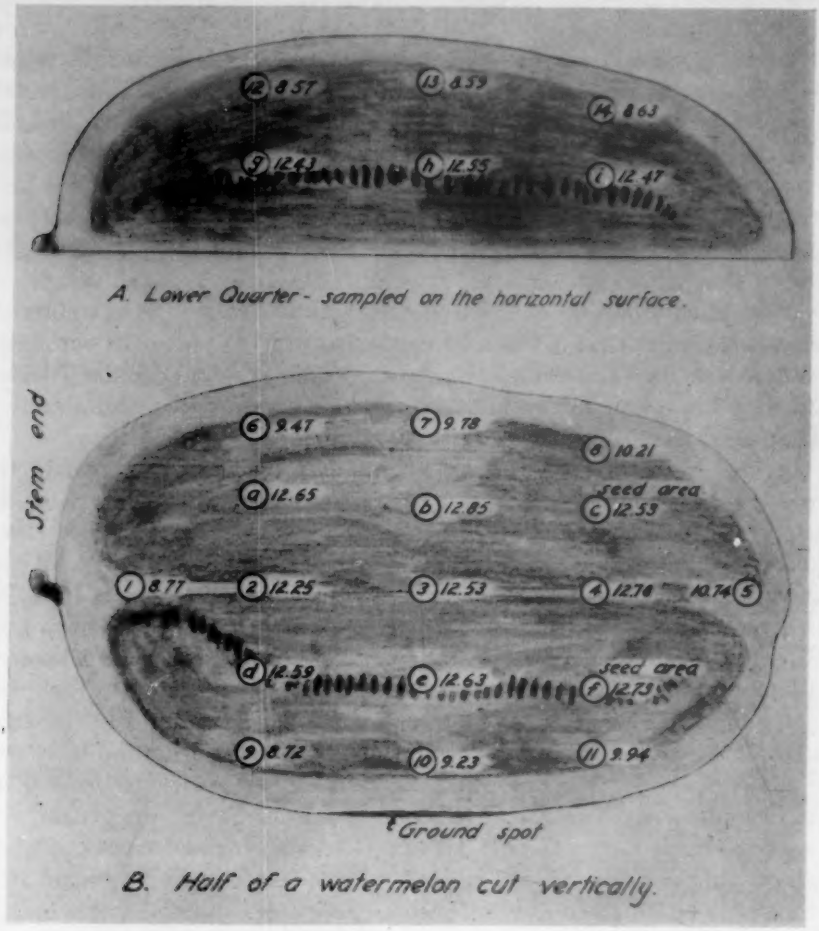

FIG. 1. The percentage of soluble solids in the different areas of a watermelon.

soluble solids. Flesh near the rind is low in soluble solids, varying from 10.74% to 8.57% and averaging 9.33%. The blossom end is almost 2% higher in solids than the stem end; likewise the samples near the upper rind are higher than those near the ground. The watermelon fruit seems to be consistently variable in soluble solids content.

To compare or improve varieties as to quality, one must have some method of obtaining an adequate sample. For some light on this problem, one may

compare the results obtained from the individual samples and the soluble solids content of the juice from the half melons. Juice from the halves averaged 11.23% soluble solids. The average for all the small samples (1–14, a–i) is 11.06%. When the average value of the melon was determined by averaging the twenty-three (fig. 1–A and 1–B) small samples and compared with the value for the half melons, there was found (by Student's method) a significant difference. Since the odds in this case were 103 to 1, evidently the twenty-three samples include too large a proportion of those near the rind. On the other hand, averaging the seventeen samples from the half (1–11, a–f) of the melon, one finds a value of 11.22%, which is not significantly different from our value for the half melon sections (11.23%). If

TABLE I

SOLUBLE SOLIDS CONTENT OF DIFFERENT SECTIONS OF A WATERMELON FRUIT

	MELON SECTION—SEED OR CENTER		MELON SECTION—NEAR RIND	
MELON	SECTION DESIGNATION, FIGURE 1	SOLUBLE SOLIDS*	SECTION DESIGNATION, FIGURE 1	SOLUBLE SOLIDS*
		%		%
Seed	B–b	12.85	B–5	10.74
Center	B–4	12.78	B–8	10.21
Seed	B–f	12.73	B–11	9.94
Seed	B–a	12.65	B–7	9.78
Seed	B–e	12.63	B–6	9.47
Seed	B–d	12.59	B–10	9.23
Seed	A–h	12.55	B–1	8.77
Center	B–3	12.53	B–9	8.72
Seed	B–c	12.53	A–14	8.63
Seed	A–i	12.47	A–13	8.59
Seed	A–g	12.43	A–12	8.57
Center	B–2	12.25		

* Arranged in descending order.

individual regions are compared within a given melon, a difference of about 0.6% soluble solids is necessary for a significant difference, with odds of 19 to 1.

None of the twenty-three areas selected had an average value of 11.23%. Twelve of the percentages are higher and eleven are lower. If, for sampling, one must choose a sector that is similar in composition to all the juice, it will have to be located between the rind and the seed region. Such a sector might lie between samples 4 and 5. Area 3, which has been used for sampling many melons, is desirable from the standpoint of the small variation from the surrounding tissue. One should remember that this area is 1.3% higher in soluble solids than the average.

In a fruit so variable as the watermelon, the choice of one area for sampling must involve certain dangers of inaccuracy. The data obtained in this experiment resemble those published by TUCKER (**4**) on the variability of the readings. According to Tucker, the center flesh varied from 8.1% to 14.9%

soluble solids, and the flesh near the rind from 4.1% to 6.8% soluble solids. SCOTT and MACGILLIVRAY (**3**) have shown a similar amount of variability in cantaloupe fruits, *Cucumis melo* L.

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NOTES

Chicago Meeting.—Plans for the annual meeting of the Society include the usual dinner to be held at the Congress Hotel which is near Headquarters at the Stevens; the price will be $3.50 per plate. In the light of present arrangements for session rooms it is imperative that all members of the Society register since the more Plant Physiologists who register, the larger the proportion of room rentals for our Society that will be paid by the A.A.A.S. Full publicity has been released relative to registration and the General Program may be obtained from the offices of the A.A.A.S. at 1515 Massachusetts Avenue, N.W., Washington 5, upon payment of the usual fee of two dollars. This program is planned for release on December 1; early registration is suggested.

Purdue Section Meeting.—On July 24 and 25, the Purdue Section of the AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS celebrated the Twenty-first Anniversary of its organization in 1926. DR. E. J. LUND, University of Texas, gave three lectures on the general subject of "Bioelectrical Phenomena and Cell Correlations" followed by a round table discussion of the results that he presented. The meetings were opened with registration during the morning of July 24 and inspection of a number of interesting projects in the biological laboratories. Forty-five people attended the noon luncheon and heard a very interesting and enlightening discussion concerning the proposed Institute of Biological Sciences by DR. J. FISHER STANFIELD. About 90 attended DR. LUND's first lecture on "Pattern and Variations of the Bioelectric Field" and the same number heard his second lecture at the dinner meeting on Thursday evening on "Orientation of Growth and Polarity of the Field." About 70 were present to listen to a discussion Friday morning on "Electrochemical Basis of the Electric Polarity of the Cell" and participated in a round table discussion following the lecture. The meetings were climaxed with a trip through Turkey Run State Park which fifty attended on Friday afternoon. Officers for next year are DR. R. E. GIRTON, Chairman and DR. H. H. KRAMER, Secretary-Treasurer.

General Botany Laboratory Book.—EDWARD M. PALMQUIST and LOREN C. PETRY. W. B. Saunders Co., Philadelphia. 174 pages. $2.25. This latest addition to the list of manuals for introductory plant science represents thirty-five exercises which are adaptable to varied types of courses. Many questions, with space for answers, provide motivation for closer study of materials; prepared drawings for labelling are ample. With a coiled wire binding it is intended that the book should be turned in for examination as a unit rather than as separate exercises; certain record sheets, however, are to be torn out and handed in. Winter keys to common trees are included in the appendix. This manual is clearly written and illustrated, represents

time-proven experiments, and should be well received as a useful guide in the laboratory.

Abdel Galil El Gawadi.—Dr. Abdel Galil El Gawadi was born May 4, 1908 at Faraskur, Egypt. Educated in Egypt, he entered Fouad I University of Cairo in 1926 and continued his education at Cambridge University, England where he received the Doctor of Philosophy degree in 1935. Here he met his future wife, Hilda Robisher, and two daughters, Aida and Nadya, were born of his marriage in 1936.

Dr. Gawadi returned to Egypt to continue his career at Fouad I University as a lecturer in Plant Physiology where he remained until 1942 when he was transferred to the new Farouk I University of Alexandria. Coming to the United States in 1946 he affiliated with the Brooklyn Botanic Garden as a Research Fellow with the general problem of Leaf Abscission. A promising career was brought to an abrupt end by his death on August 21, 1947.

While Dr. Gawadi had enjoyed only limited opportunities to enlarge his circle of friends in this country he was highly esteemed by his colleagues. Clarity of vision, a cordial manner, and scrupulous honesty were attributes enjoyed and respected by all who knew him.

Radioactive Tracers in Biology: An Introduction to Tracer Methodology.—Martin D. Kamen. Academic Press Inc., New York, 1947. 281 pages. $5.80. This manual reviews concepts in nuclear physics which are basic to proper application of tracer methods in biology and provides a critical survey of existing procedures with potentialities and limitations thereof for general biological research. The author presents the actual data of his own and many other original investigations employing radioactive isotopes. Reference is also made to the use of stable isotopes in tracer studies though somewhat briefly in the interest of clarity. A survey of special topics, concerning medical applications of radioactive isotopes is included in the final section of the book.

Though this book is not a compendium for experts, it provides a thorough introduction to tracer research in biology and will be of inestimable value to biochemists, physiologists, and physicians seeking orientation tracer methodology and peaceful applications of nuclear energy. The text includes discussion of atomic nuclei, radioactive isotopes and methods of their production, radiation characteristics of tracer atoms and procedures for assay thereof, followed by comments on radioactive forms of hydrogen, carbon, phosphorus, sulphur, the light and heavy metals. The author's style is lucid, the contents are very coherently organized and the text is generously illustrated. There are numerous yet carefully selected citations in extension as well as in support of the text, enabling those who desire more extended knowledge of current aspects of radiobiology to obtain ready access to the most authoritative and recent publications in the field. Author and subject

indices make the contents available for rapid reference. The book serves a most useful purpose for biologists in collation and evaluation of a widely scattered literature as this relates to theory, preparation, testing and results in tracer research. The author and publisher are to be commended on the timeliness and general excellence of the book.

Fatty Acids, Their Chemistry and Physical Properties.—K. S. MARKLEY. Interscience Publishers, 215 Fourth Avenue, New York 3. 668 pages. $10.00. This monograph deals with the chemistry and technology of fats, oils, and related substances. The subject matter is well organized and brings together in an accessible form many data pertaining to the chemical reactions and physical properties of the fatty acids, with particular stress on the long chain fatty acids. With preliminary chapters on the history, classification, nomenclature, and isomerism of fats, waxes, and fatty acids, subsequent chapters discuss physical properties, chemical reactions, synthesis, isolation, and identification of fatty acids. Many new aspects of spectral properties, pyrolysis, autoxidation, and the biosynthesis of fatty acids are presented.

Although the book is principally intended for chemists much vital information will be of interest to the plant physiologist, particularly the section on synthesis in plants. The excellent organization of the material will obviate much laborious search of the literature for those interested in this type of research.

Bioelectric Fields and Growth.—E. J. LUND and COLLABORATORS. University of Texas Press, Austin, Texas. 391 pages. $7.50. This book collates the major research of the laboratories of physiology and biophysics at the University of Texas. It is a very helpful interpretation of experimentation by the outstanding leaders of the rapidly expanding field of electrophysiology.

Theoretical aspects of the subject matter are closely integrated with the experimental data, presented under seven separate headings. The first considers the topic of Electric Fields of the Cell and Polar Cell Aggregates, the second and third deal with the Effects of Gravity on the Electric Correlation Field in the Oat Coleoptile and the Spontaneous Variations in the Electric Field Potentials of the Root of the Onion. The subsequent sections treat the topics of Polar Bioelectric Fields and Polar Growth Under Conditions of Applied Electric Fields, Biocoulometry, General Discussion of the Results and Significance of the Results by the senior author. The final section consists of a concise bibliography of Continuous Bioelectric Currents and Bioelectric Fields in Animals and Plants with index by H. F. Rosene.

Acta Scandinavica.—Volume I, 1947. Published by Chemical Societies in Denmark, Finland, Norway, and Sweden. Distributed by Einor Munksgaard, Copenhagen, Denmark. Annual Subscription, $8.50 plus postage.

The Journal Acta Chemica Scandinavica is again readily available to American subscribers. Dealing with the work of Scandinavian chemists in the fields of general, physical, inorganic, organic, and biochemistry, this publication appears ten times yearly. All articles and contributions are printed in one of three languages: English, French, or German. Aquaintanceship with this journal should be especially helpful to the American plant physiologist in keeping abreast with recent research of the Scandinavian countries.

Advances in Enzymology and Related Subjects of Biochemistry.—Volume 7, 1947. Edited by F. F. NORD, Interscience Publishers, 215 Fourth Avenue, New York 3. 665 pages. $8.75. Of special interest to physiologists in the newest volume of this distinguished series will be the sections on: Permeability and Enzyme Reactions by S. C. BROOKS; Properties of Protoplasm with Special Reference to the Influence of Enzyme Reactions by WILLIAM SEIFRIZ; Applications of Radioactive Indicaters in Turnover Studies by G. HEVSEY of Stockholm; Bacterial Luminescence by FRANK H. JOHNSON; Heme-Linked Groups and Mode of Action of Some Hemoproteins by HUGO THEORELL of Stockholm; Interrelations in Microorganisms between Growth and the Metabolism of Vitamin-like Substances from Fungi and Green Plants by FREDERICK KAVANAGH; and Recent Progress in Industrial Fermentations by F. M. HILDEBRANDT. This volume also contains a cumulative index to Volumes I to VII as well as author and subject indices to Volume VII.

Annual Review of Biochemistry.—Volume XVI, 1947. Annual Reviews, Inc. Stanford University Postoffice, California. 740 pages. $6.00. Of the twenty-five sections of the most recent volume of this review journal, the following will be of particular interest to physiologists: Biological Oxidations and Reductions; Proteolytic Enzymes; Metabolism of Lipids, Carbohydrates, Proteins and Amino Acids; Antioxidants; Nitrogenous Constituents of Plants; Mineral Nutrition of Plants; Growth Substances in Higher Plants and Bacterial Metabolism. The volume contains complete, cross-referenced author and subject indices.

Plant and Soil.—Volume I, 1947. E. G. MULDER, Editor. A new periodical published by Martinus Nijhoff Company, The Hague, Holland. 400 pages. Subscription $7.50 per volume. A new important international periodical, "Plant and Soil," will be issued towards the end of 1947. It is devoted to the study of plant nutrition, plant chemistry, and the related subjects of soil science, soil microbiology, and soil-borne plant diseases. Realizing that the increasing demand for intensive crop production all over the world necessitates a quick exchange of the results of scientific research between the workers in this field, a number of outstanding scientists from different countries have decided to collaborate in establishing this new journal.

Among the Editors we read the names of GÄUMANN of Zürich; LUNDE-

GÅRDH, PENNINGBY, and MELIN of Uppsala; HOAGLAND of Berkeley; KONINGSBERGER of Utrecht; WAKSMAN of New Brunswick; WESTERDIJK of Baarn; VERTANEN of Helsinki, and many other outstanding scientists. Original contributions in English, French or German may be sent to the secretary of the Board of Editors, DR. E. G. MULDER, Agricultural Experiment Station, Eemskanaal ZZ. 1, Groningen, Holland.

Statistical Analysis of Biology.—K. MATHER. Interscience Publishers, 215 Fourth Avenue, New York 3. 267 pages. $5.00. This book is a valuable analytical tool to plant scientists and research workers in general. The author outlines the scope of the various statistical methods and their interrelationship. Representative analyses and examples in the text show how statistical evaluations are applicable to the data of the biological sciences. The subject matter dealing with such basic principles of statistics as Probability and Significance, Distributions, Tests of Significance, The Interrelationship of Two Variables, Polynomial and Multiple Regressions, and Correlations is couched in a language readily understood by the nonmathematician. A glossary of terms also facilitates acquaintanceship with the vocabulary and mathematical symbols used in statistics. The volume consists of 13 chapters with an individual list of references for each subject. A set of conversion tables and a general index are also included.

Methods of Vitamin Assay.—Prepared and Edited by the Association of Vitamin Chemists, Inc. 1947. Interscience Publishers, 215 Fourth Avenue, New York 3. 189 pages. $3.50. This monograph meets the need for a concise yet critical compilation of standardized methods of vitamin analysis. Methods are presented for analysis of foods, feedstuffs, pharmaceutical, and biological products. The most accurate and widespread methods for determination of the major vitamins are listed along with selected references to other methods. Separate bibliographies pertinent to the particular subject are included with each individual chapter.

Technological and Physical Investigations on Natural and Synthetic Rubbers.—A. G. WILDSCHUT. 1946. Elseveir Publishing Company, Inc., New York. 173 pages. $3.00. This book is one of a series of monographs on the progress of research in Holland during the war. The subject matter comprises technological investigations and physical investigations on natural and artificial rubbers and related components as carried on in the Netherlands from 1939 to 1944. Though the book is intended primarily as a reference for rubber technologists, this volume will be of interest and use to plant scientists as a compilation of much important research on rubber carried on during World War II.

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